



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> : <b>C12N 15/03, 15/05, A01N 13/00, A61K 39/00, 1/11, 1/15, A01H 4/00</b>		<b>A1</b>	(11) International Publication Number: <b>WO 98/32844</b>
			(43) International Publication Date: 30 July 1998 (30.07.98)
(21) International Application Number: PCT/US98/01507 (22) International Filing Date: 27 January 1998 (27.01.98) (30) Priority Data: 60/036,048 27 January 1997 (27.01.97) US (71) Applicant: CORNELL RESEARCH FOUNDATION, INC. [US/US]; Suite 105, 20 Thornwood Drive, Ithaca, NY 14850 (US). (72) Inventors: QIU, Dewen; 17815 8th Avenue, N.E., Seattle, WA 98155 (US). WEI, Zhong-Min; 8230 N.E. 125th Court, Kirkland, WA 98034 (US). BEER, Steven, V.; 211 Hudson Street, Ithaca, NY 14850 (US). (74) Agents: GOLDMAN, Michael, L. et al.; Nixon, Hargrave, Devans & Doyle LLP, Clinton Square, P.O. Box 1051, Rochester, NY 14603 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.                  Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
(54) Title: ENHANCEMENT OF GROWTH IN PLANTS			
(57) Abstract			
<p>The present invention relates to a method of enhancing growth of plants. This involves applying a hypersensitive response elicitor polypeptide or protein in a non-infectious form to a plant or plant seed under conditions effective to enhance growth of the plant or plants produced from the plant seed. Alternatively, transgenic plants or transgenic plant seeds transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein can be provided and the transgenic plants or plants resulting from the transgenic plant seeds are grown under conditions effective to enhance plant growth.</p>			

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

## ENHANCEMENT OF GROWTH IN PLANTS

This application claims the benefit of U.S. Provisional Patent Application Serial No. 60/036,048, filed January 27, 1997.

This invention was made with support from the  
5 U.S. Government under USDA NRI Competitive Research Grant  
No. 91-37303-6430.

### FIELD OF THE INVENTION

10 The present invention relates to the  
enhancement of growth in plants.

### BACKGROUND OF THE INVENTION

15 The improvement of plant growth by the  
application of organic fertilizers has been known and  
carried out for centuries (H. Marschner, "Mineral  
Nutrition of Higher Plants," Academic Press: New York  
pg. 674 (1986). Modern man has developed a complex  
20 inorganic fertilizer production system to produce an easy  
product that growers and farmers can apply to soils or  
growing crops to improve performance by way of growth  
enhancement. Plant size, coloration, maturation, and  
yield may all be improved by the application of  
25 fertilizer products. Inorganic fertilizers include such  
commonly applied chemicals as ammonium nitrate. Organic  
fertilizers may include animal manures and composted lawn  
debris, among many other sources.

In most recent years, researchers have sought  
30 to improve plant growth through the use of biological  
products. Insect and disease control agents such as  
*Beauveria bassiana* and *Trichoderma harizamum* have been  
registered for the control of insect and disease problems  
and thereby indirectly improve plant growth and  
35 performance (Fravel et al., "Formulation of

- 2 -

Microorganisms to Control Plant Diseases," Formulation of Microbial Biopesticides, Beneficial Microorganisms, and Nematodes, H.D. Burges, ed. Chapman and Hall: London (1996).

5           There is some indication of direct plant growth enhancement by way of microbial application or microbial by-products. Nodulating bacteria have been added to seeds of leguminous crops when introduced to a new site (Weaver et al., "Rhizobium," Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties, 2nd ed., 10   American Society of Agronomy: Madison (1982)). These bacteria may improve the nodulation efficiency of the plant and thereby improve the plant's ability to convert free nitrogen into a usable form, a process called 15   nitrogen fixation. Non-leguminous crops do not, as a rule, benefit from such treatment. Added bacteria such as *Rhizobium* directly parasitize the root hairs, then begin a mutualistic relationship by providing benefit to the plant while receiving protection and sustenance.

20           Mycorrhizal fungi have also been recognized as necessary microorganisms for optional growth of many crops, especially conifers in nutrient-depleted soils. Mechanisms including biosynthesis of plant hormones (Frankenberger et al., "Biosynthesis of Indole-3-Acetic 25   Acid by the Pine Ectomycorrhizal Fungus *Pisolithus tinctorius*," Appl. Environ. Microbiol. 53:2908-13 (1987)), increased uptake of minerals (Harley et al., "The Uptake of Phosphate by Excised Mycorrhizal Roots of Beech," New Phytologist 49:388-97 (1950) and Harley et 30   al., "The Uptake of Phosphate by Excised Mycorrhizal Roots of Beech. IV. The Effect of Oxygen Concentration Upon Host and Fungus," New Phytologist 52:124-32 (1953)), and water (A.B. Hatch, "The Physical Basis of Mycotrophy in *Pinus*," Black Rock Forest Bull. No. 6, 168 pp. (1937)) 35   have been postulated. Mycorrhizal fungi have not

- 3 -

achieved the common frequency of use that nodulating bacteria have due to variable and inconsistent results with any given mycorrhizal strain and the difficulty of study of the organisms.

- 5 Plant growth-promoting rhizobacteria ("PGPR") have been recognized in recent years for improving plant growth and development. Hypothetical mechanisms range from direct influences (e.g., increased nutrient uptake) to indirect mechanisms (e.g., pathogen displacement).
- 10 Growth enhancement by application of a PGPR generally refers to inoculation with a live bacterium to the root system and achieving improved growth through bacterium-produced hormonal effects, siderophores, or by prevention of disease through antibiotic production, or competition.
- 15 In all of the above cases, the result is effected through root colonization, sometimes through the application of seed coatings. There is limited information to suggest that some PGPR strains may be direct growth promoters that enhance root elongation under gnotobiotic conditions
- 20 (Anderson et al., "Responses of Bean to Root Colonization With *Pseudomonas putida* in a Hydroponic System," Phytopathology 75:992-95 (1985), Lifshitz et al., "Growth Promotion of Canola (rapeseed) Seedlings by a Strain of *Pseudomonas putida* Under Gnotobiotic Conditions," Can. J. Microbiol. 33:390-95 (1987), Young et al., "PGPR: Is
- 25 There Relationship Between Plant Growth Regulators and the Stimulation of Plant Growth or Biological Activity?," Promoting Rhizobacteria: Progress and Prospects, Second International Workshop on Plant Growth-promoting
- 30 Rhizobacteria, pp. 182-86 (1991), Loper et al., "Influence of Bacterial Sources of Indole-3-Acetic Acid on Root Elongation of Sugar Beet," Phytopathology 76:386-89 (1986), and Müller et al., "Hormonal Interactions in the Rhizosphere of Maize (*Zea mays* L.) and Their Effect
- 35 on Plant Development," Z. Pflanzenernährung Bodenkunde

- 4 -

- 152:247-54 (1989); however, the production of plant growth regulators has been proposed as the mechanism mediating these effects. Many bacteria produce various plant growth regulators *in vitro* (Atzorn et al.,
- 5 "Production of Gibberellins and Indole-3-Acetic Acid by *Rhizobium phaseoli* in Relation to Nodulation of *Phaseolus vulgaris* Roots," Planta 175:532-38 (1988) and M. E. Brown, "Plant Growth Substances Produced by Micro-Organism of Solid and Rhizosphere," J. Appl. Bact.
- 10 35:443-51 (1972)) or antibiotics (Gardner et al., "Growth Promotion and Inhibition by Antibiotic-Producing Fluorescent *Pseudomonads* on Citrus Roots," Plant Soil 77:103-13 (1984)). Siderophore production is another mechanism proposed for some PGPR strains (Ahl et al.,
- 15 "Iron Bound-Siderophores, Cyanic Acid, and Antibiotics Involved in Suppression of *Thievaliopsis basicola* by a *Pseudomonas fluorescens* Strain," J. Phytopathol. 116:121-34 (1986), Kloepper et al., "Enhanced Plant Growth by Siderophores Produced by Plant Growth-Promoting
- 20 Rhizobacteria," Nature 286:885-86 (1980), and Kloepper et al., "*Pseudomonas siderophores*: A Mechanism Explaining Disease-Suppressive Soils," Curr. Microbiol. 4:317-20 (1980)). The colonization of root surfaces and thus the direct competition with pathogenic bacteria on the
- 25 surfaces is another mechanism of action (Kloepper et al., "Relationship of *in vitro* Antibiosis of Plant Growth-Promoting Rhizobacteria to Plant Growth and the Displacement of Root Microflora," Phytopathology 71:1020-24 (1981), Weller, et al., "Increased Growth of Wheat by
- 30 Seed Treatments With Fluorescent *Pseudomonads*, and Implications of *Pythium* Control," Can. J. Microbiol. 8:328-34 (1986), and Suslow et al., "Rhizobacteria of Sugar Beets: Effects of Seed Application and Root Colonization on Yield," Phytopathology 72:199-206
- 35 (1982)). Canola (rapeseed) studies have indicated PGPR

- 5 -

- increased plant growth parameters including yields, seedling emergence and vigor, early-season plant growth (number of leaves and length of main runner), and leaf area (Kloepper et al., "Plant Growth-Promoting Rhizobacteria on Canola (rapeseed)," Plant Disease 72:42-46 (1988)). Studies with potato indicated greater yields when *Pseudomonas* strains were applied to seed potatoes (Burr et al., "Increased Potato Yields by Treatment of Seed Pieces With Specific Strains of *Pseudomonas* *Fluorescens* and *P. putida*," Phytopathology 68:1377-83 (1978), Kloepper et al., "Effect of Seed Piece Inoculation With Plant Growth-Promoting Rhizobacteria on Populations of *Erwinia carotovora* on Potato Roots and in Daughter Tubers," Phytopathology 73:217-19 (1983), Geels et al., "Reduction of Yield Depressions in High Frequency Potato Cropping Soil After Seed Tuber Treatments With Antagonistic Fluorescent *Pseudomonas* spp.," Phytopathol. Z. 108:207-38 (1983), Howie et al., "Rhizobacteria: Influence of Cultivar and Soil Type on Plant Growth and Yield of Potato," Soil Biol. Biochem. 15:127-32 (1983), and Vransy et al., "Growth and Yield of Potato Plants Inoculated With Rhizosphere Bacteria," Folia Microbiol. 29:248-53 (1984)). Yield increase was apparently due to the competitive effects of the PGPR to eliminate pathogenic bacteria on the seed tuber, possibly by antibiosis (Kloepper et al., "Effect of Seed Piece Inoculation With Plant Growth-Promoting Rhizobacteria on Populations of *Erwinia carotovora* on Potato Roots and in Daughter Tubers," Phytopathology 73:217-19 (1983), Kloepper et al., "Effects of Rhizosphere Colonization by Plant Growth-Promoting Rhizobacteria on Potato Plant Development and Yield," Phytopathology 70:1078-82 (1980), Kloepper et al., "Emergence-Promoting Rhizobacteria: Description and Implications for Agriculture," pp. 155-164, Iron, Siderophores, and Plant Disease, T.R.

- 6 -

Swinburne, ed. Plenum, New York (1986), and Kloepper et al., "Relationship of *in vitro* Antibiosis of Plant Growth-Promoting Rhizobacteria to Plant Growth and the Displacement of Root Microflora," Phytopathology 71:1020-24 (1981)). In several studies, plant emergence was improved using PGPR (Tipping et al., "Development of Emergence-Promoting Rhizobacteria for Supersweet Corn," Phytopathology 76:938-41 (1990) (abstract) and Kloepper et al., "Emergence-Promoting Rhizobacteria: Description and Implications for Agriculture," pp. 155-164, Iron, Siderophores, and Plant Disease, T.R. Swinburne, ed. Plenum, New York (1986)). Numerous other studies indicated improved plant health upon treatment with rhizobacteria, due to biocontrol of plant pathogens (B. Schippers, "Biological Control of Pathogens With Rhizobacteria," Phil. Trans. R. Soc. Lond. B. 318:283-93 (1988), Schroth et al., "Disease-Suppressive Soil and Root-Colonizing Bacteria," Science 216:1376-81 (1982), Stutz et al., "Naturally Occurring Fluorescent Pseudomonads Involved in Suppression of Black Root Rot of Tobacco," Phytopathology 76:181-85 (1986), and D.M. Weller, "Biological Control of Soilborne Plant Pathogens in the Rhizosphere With Bacteria," Annu. Rev. Phytopathol. 26:379-407 (1988)).

Pathogen-induced immunization of a plant has been found to promote growth. Injection of *Peronospora tabacina* externally to tobacco xylem not only alleviated stunting but also promoted growth and development. Immunized tobacco plants, in both greenhouse and field experiments, were approximately 40% taller, had a 40% increase in dry weight, a 30% increase in fresh weight, and 4-6 more leaves than control plants (Tuzun, S., et al., "The Effect of Stem Injection with *Peronospora tabacina* and Metalaxyl Treatment on Growth of Tobacco and Protection Against Blue Mould in the Field,"



- 7 -

Phytopathology, 74:804 (1984). These plants flowered approximately 2-3 weeks earlier than control plants (Tuzun, S., et al., "Movement of a Factor in Tobacco Infected with *Peronospora tabacina* Adam which Systemically Protects Against Blue Mould," Physiological Plant Pathology, 26:321-30 (1985)).

The present invention is directed to an improvement over prior plant growth enhancement procedures.

10

#### SUMMARY OF THE INVENTION

The present invention relates to a method of enhancing growth in plants. This method involves applying a hypersensitive response elicitor polypeptide or protein in a non-infectious form to plants or plant seeds under conditions to impart enhanced growth to the plants or to plants grown from the plant seeds.

As an alternative to applying a hypersensitive response elicitor polypeptide or protein to plants or plant seeds in order to impart enhanced growth to the plants or to plants grown from the seeds, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the plant under conditions effective to permit that DNA molecule to enhance growth. Alternatively, a transgenic plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein can be provided and planted in soil. A plant is then propagated from the planted seed under conditions effective to permit that DNA molecule to enhance growth.

- 8 -

The present invention is directed to effecting any form of plant growth enhancement or promotion. This can occur as early as when plant growth begins from seeds or later in the life of a plant. For example, plant

5 growth according to the present invention encompasses greater yield, increased quantity of seeds produced, increased percentage of seeds germinated, increased plant size, greater biomass, more and bigger fruit, earlier fruit coloration, and earlier fruit and plant maturation.

10 As a result, the present invention provides significant economic benefit to growers. For example, early germination and early maturation permit crops to be grown in areas where short growing seasons would otherwise preclude their growth in that locale. Increased

15 percentage of seed germination results in improved crop stands and more efficient seed use. Greater yield, increased size, and enhanced biomass production allow greater revenue generation from a given plot of land. It is thus apparent that the present invention constitutes a

20 significant advance in agricultural efficiency.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a map of plasmid vector pCPP2139

25 which contains the *Erwinia amylovora* hypersensitive response elicitor gene.

Figure 2 is a map of plasmid vector pCPP50 which does not contain the *Erwinia amylovora* hypersensitive response elicitor gene but is otherwise

30 the same as plasmid vector pCPP2139 shown in Figure 1. See Masui, et al., Bio/Technology 2:81-85 (1984), which is hereby incorporated by reference.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method of enhancing growth in plants. This method involves  
5 applying a hypersensitive response elicitor polypeptide or protein in a non-infectious form to all or part of a plant or a plant seed under conditions to impart enhanced growth to the plant or to a plant grown from the plant seed. Alternatively, plants can be treated in this  
10 manner to produce seeds, which when planted, impart enhanced growth in progeny plants.

As an alternative to applying a hypersensitive response elicitor polypeptide or protein to plants or plant seeds in order to impart enhanced growth to the  
15 plants or to plants grown from the seeds, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein  
20 and growing the plant under conditions effective to permit that DNA molecule to enhance growth. Alternatively, a transgenic plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein can be provided and planted in  
25 soil. A plant is then propagated from the planted seed under conditions effective to permit that DNA molecule to enhance growth.

The hypersensitive response elicitor polypeptide or protein utilized in the present invention  
30 can correspond to hypersensitive response elicitor polypeptides or proteins derived from a wide variety of fungal and bacterial pathogens. Such polypeptides or proteins are able to elicit local necrosis in plant tissue contacted by the elicitor.

- 10 -

Examples of suitable bacterial sources of polypeptide or protein elicitors include *Erwinia*, *Pseudomonas*, and *Xanthomonas* species (e.g., the following bacteria: *Erwinia amylovora*, *Erwinia chrysanthemi*,  
5 *Erwinia stewartii*, *Erwinia carotovora*, *Pseudomonas syringae*, *Pseudomonas solanacearum*, *Xanthomonas campestris*, and mixtures thereof).

An example of a fungal source of a hypersensitive response elicitor protein or polypeptide  
10 is *Phytophthora*. Suitable species of *Phytophthora* include *Phytophthora pythium*, *Phytophthora cryptogea*, *Phytophthora cinnamomi*, *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora*.

The embodiment of the present invention where  
15 the hypersensitive response elicitor polypeptide or protein is applied to the plant or plant seed can be carried out in a number of ways, including: 1) application of an isolated elicitor polypeptide or protein; 2) application of bacteria which do not cause  
20 disease and are transformed with genes encoding a hypersensitive response elicitor polypeptide or protein; and 3) application of bacteria which cause disease in some plant species (but not in those to which they are applied) and naturally contain a gene encoding the  
25 hypersensitive response elicitor polypeptide or protein. In addition, seeds in accordance with the present invention can be recovered from plants which have been treated with a hypersensitive response elicitor protein or polypeptide in accordance with the present invention.

30 In one embodiment of the present invention, the hypersensitive response elicitor polypeptides or proteins can be isolated from their corresponding organisms and applied to plants or plant seeds. Such isolation procedures are well known, as described in  
35 Arlat, M., F. Van Gijsegem, J. C. Huet, J. C. Pemollet,

- 11 -

and C. A. Boucher, "PopA1, a Protein which Induces a Hypersensitive-like Response in Specific Petunia Genotypes is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-553 (1994); He, S. Y., H. C. Huang, and A. Collmer, "*Pseudomonas syringae* pv. *syringae* Harpin<sub>PS</sub>: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-1266 (1993); and Wei, Z.-M., R. J. Laby, C. H. Zumoff, D. W. Bauer, S.-Y. He, A. Collmer, and S. V. Beer, "Harpin Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which are hereby incorporated by reference. See also pending U.S. Patent Application Serial Nos. 08/200,024 and 08/062,024, which are hereby incorporated by reference. Preferably, however, the isolated hypersensitive response elicitor polypeptides or proteins of the present invention are produced recombinantly and purified as described below.

In other embodiments of the present invention, the hypersensitive response elicitor polypeptide or protein of the present invention can be applied to plants or plant seeds by applying bacteria containing genes encoding the hypersensitive response elicitor polypeptide or protein. Such bacteria must be capable of secreting or exporting the polypeptide or protein so that the elicitor can contact plant or plant seeds cells. In these embodiments, the hypersensitive response elicitor polypeptide or protein is produced by the bacteria in planta or on seeds or just prior to introduction of the bacteria to the plants or plant seeds.

In one embodiment of the bacterial application mode of the present invention, the bacteria do not cause the disease and have been transformed (e.g., recombinantly) with genes encoding a hypersensitive response elicitor polypeptide or protein. For example,

- 12 -

*E. coli*, which does not elicit a hypersensitive response in plants, can be transformed with genes encoding a hypersensitive response elicitor polypeptide or protein and then applied to plants. Bacterial species other than  
 5 *E. coli* can also be used in this embodiment of the present invention.

In another embodiment of the bacterial application mode of the present invention, the bacteria do cause disease and naturally contain a gene encoding a  
 10 hypersensitive response elicitor polypeptide or protein. Examples of such bacteria are noted above. However, in this embodiment, these bacteria are applied to plants or their seeds which are not susceptible to the disease carried by the bacteria. For example, *Erwinia amylovora*  
 15 causes disease in apple or pear but not in tomato. However, such bacteria will elicit a hypersensitive response in tomato. Accordingly, in accordance with this embodiment of the present invention, *Erwinia amylovora* can be applied to tomato plants or seeds to enhance  
 20 growth without causing disease in that species.

The hypersensitive response elicitor polypeptide or protein from *Erwinia chrysanthemi* has an amino acid sequence corresponding to SEQ. ID. No. 1 as follows:

25

Met Gln Ile Thr Ile Lys Ala His Ile Gly Gly Asp Leu Gly Val Ser  
 1 5 10 15

30

Gly Leu Gly Ala Gln Gly Leu Lys Gly Leu Asn Ser Ala Ala Ser Ser  
 20 25 30

Leu Gly Ser Ser Val Asp Lys Leu Ser Ser Thr Ile Asp Lys Leu Thr  
 35 40 45

35

Ser Ala Leu Thr Ser Met Met Phe Gly Gly Ala Leu Ala Gln Gly Leu  
 50 55 60

40

Gly Ala Ser Ser Lys Gly Leu Gly Met Ser Asn Gln Leu Gly Gln Ser  
 65 70 75 80

Phe Gly Asn Gly Ala Gln Gly Ala Ser Asn Leu Leu Ser Val Pro Lys  
 85 90 95

- 13 -

	Ser Gly Gly Asp Ala Leu Ser Lys Met Phe Asp Lys Ala Leu Asp Asp	100	105	110
5	Leu Leu Gly His Asp Thr Val Thr Lys Leu Thr Asn Gln Ser Asn Gln	115	120	125
	Leu Ala Asn Ser Met Leu Asn Ala Ser Gln Met Thr Gln Gly Asn Met	130	135	140
10	Asn Ala Phe Gly Ser Gly Val Asn Asn Ala Leu Ser Ser Ile Leu Gly	145	150	155
	Asn Gly Leu Gly Gln Ser Met Ser Gly Phe Ser Gln Pro Ser Leu Gly	165	170	175
15	Ala Gly Gly Leu Gln Gly Leu Ser Gly Ala Gly Ala Phe Asn Gln Leu	180	185	190
	Gly Asn Ala Ile Gly Met Gly Val Gly Gln Asn Ala Ala Leu Ser Ala	195	200	205
20	Leu Ser Asn Val Ser Thr His Val Asp Gly Asn Asn Arg His Phe Val	210	215	220
	Asp Lys Glu Asp Arg Gly Met Ala Lys Glu Ile Gly Gln Phe Met Asp	225	230	235
25	Gln Tyr Pro Glu Ile Phe Gly Lys Pro Glu Tyr Gln Lys Asp Gly Trp	245	250	255
30	Ser Ser Pro Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser Lys	260	265	270
	Pro Asp Asp Asp Gly Met Thr Gly Ala Ser Met Asp Lys Phe Arg Gln	275	280	285
35	Ala Met Gly Met Ile Lys Ser Ala Val Ala Gly Asp Thr Gly Asn Thr	290	295	300
	Asn Leu Asn Leu Arg Gly Ala Gly Gly Ala Ser Leu Gly Ile Asp Ala	305	310	315
40	Ala Val Val Gly Asp Lys Ile Ala Asn Met Ser Leu Gly Lys Leu Ala	325	330	335
45	Asn Ala			

This hypersensitive response elicitor polypeptide or  
 50 protein has a molecular weight of 34 kDa, is heat stable,  
 has a glycine content of greater than 16%, and contains  
 substantially no cysteine. The *Erwinia chrysanthemi*  
 hypersensitive response elicitor polypeptide or protein  
 is encoded by a DNA molecule having a nucleotide sequence  
 55 corresponding to SEQ. ID. No. 2 as follows:

- 14 -

	CGATTTTACC CGGGTGAACG TGCTATGACC GACAGCATCA CGGTATTCGA CACCGTTACG	60
	GCGTTTATGG CCGCGATGAA CCGGCATCAG GCGGCGCGCT GGTCGCCGCA ATCCGGCGTC	120
5	GATCTGGTAT TTCAGTTTGG GGACACCGGG CGTGAACCTCA TGATGCAGAT TCAGCCGGGG	180
	CAGCAATATC CCGGCATGTT GCGCACGCTG CTCGCTCGTC GTTATCAGCA GCGGCGAGAG	240
	TGCGATGGCT GCCATCTGTG CCTGAACGGC AGCGATGTAT TGATCCTCTG GTGGCCGCTG	300
10	CCGTCGGATC CCGGCAGTTA TCCGCAGGTG ATCGAACGTT TGTTTGAACCT GGCGGGAATG	360
	ACGTTGCCGT CGCTATCCAT AGCACCGACG GCGCGTCCGC AGACAGGGAA CCGACGCGCC	420
15	CGATCATTAA GATAAAGGCG GCTTTTTTTTA TTGCAAAACG GTAACGGTGA GGAACCGTTT	480
	CACCGTCGGC GTCACCTAGT AACAACTATC CATCATGATG CCTACATCGG GATCGGCGTG	540
	GGCATCCGTT GCAGATACTT TTGCGAACAC CTGACATGAA TGAGGAAACG AAATTATGCA	600
20	AATTACGATC AAAGCGCACA TCGGCGGTGA TTTGGGCGTC TCCGGTCTGG GGCTGGGTGC	660
	TCAGGGACTG AAAGGACTGA ATTCCGCGGC TTCATCGCTG GGTCCAGCG TGGATAAACT	720
25	GAGCAGCACC ATCGATAAGT TGACCTCCGC GCTGACTTCG ATGATGTTTG GCGGCGCGCT	780
	GGCGCAGGGG CTGGGCGCCA GCTCGAAGGG GCTGGGGATG AGCAATCAAC TGGGCCAGTC	840
	TTTCGGCAAT GGCGCGCAGG GTGCGAGCAA CCTGCTATCC GTACCGAAAT CCGGCGGCGA	900
30	TGCGTTGTCA AAAATGTTTG ATAAAGCGCT GGACGATCTG CTGGGTCATG ACACCGTGAC	960
	CAAGCTGACT AACCAGAGCA ACCAACTGGC TAATTCAATG CTGAACGCCA GCCAGATGAC	1020
35	CCAGGGTAAT ATGAATGCGT TCGGCAGCGG TGTGAACAAC GCACTGTCGT CCATTCTCGG	1080
	CAACGGTCTC GGCCAGTCGA TGAGTGGCTT CTCTCAGCCT TCTCTGGGGG CAGGCGGCTT	1140
	GCAGGGCCTG AGCGGCGCGG GTGCATTCAA CCAGTTGGGT AATGCCATCG GCATGGGCGT	1200
40	GGGCGAGAAT GCTGCGCTGA GTGCGTTGAG TAACGTCAGC ACCCACGTAG ACGGTAACAA	1260
	CCGCCACTTT GTAGATAAAG AAGATCGCGG CATGGCGAAA GAGATCGGCC AGTTTATGGA	1320
45	TCAGTATCCG GAAATATTCT GTAAACCGGA ATACCAGAAA GATGGCTGGA GTTCGCCGAA	1380
	GACGGACGAC AAATCCTGGG CTAAAGCGCT GAGTAAACCG GATGATGACG GTATGACCGG	1440



- 15 -

CGCCAGCATG GACAAATTCC GTCAGGCGAT GGGTATGATC AAAAGCGCGG TGGCGGGTGA 1500

TACCGGCAAT ACCAACCTGA ACCTGCGTGG CGCGGGCGGT GCATCGCTGG GTATCGATGC 1560

5 GGCTGTCGTC GGCATAAAA TAGCCAACAT GTCGCTGGGT AAGCTGGCCA ACGCCTGATA 1620

ATCTGTGCTG GCCTGATAAA GCGGAAACGA AAAAAGAGAC GGGGAAGCCT GTCTCTTTTC 1680

TTATTATGCG GTTTATGCGG TTACCTGGAC CGGTTAATCA TCGTCATCGA TCTGGTACAA 1740

10 ACGCACATTT TCCCGTTCAT TCGCGTCGTT ACGCGCCACA ATCGCGATGG CATCTTCCTC 1800

GTCGCTCAGA TTGCGCGGCT GATGGGGAAC GCCGGGTGGA ATATAGAGAA ACTCGCCGGC 1860

15 CAGATGGAGA CACGTCTGCG ATAAATCTGT GCCGTAACGT GTTCTATCC GCCCCTTAG 1920

CAGATAGATT GCGGTTTCGT AATCAACATG GTAATGCGGT TCCGCCTGTG CGCCGGCCGG 1980

GATCACCACA ATATTCATAG AAAGCTGTCT TGCACCTACC GTATCGCGGG AGATACCGAC 2040

20 AAAATAGGGC AGTTTTTGCG TGGTATCCGT GGGGTGTTCC GGCCTGACAA TCTTGAGTTG 2100

GTCGTCATC ATCTTTCTCC ATCTGGGCGA CCTGATCGGT T 2141

25 The hypersensitive response elicitor  
polypeptide or protein derived from *Erwinia amylovora* has  
an amino acid sequence corresponding to SEQ. ID. No. 3 as  
follows:

30 Met Ser Leu Asn Thr Ser Gly Leu Gly Ala Ser Thr Met Gln Ile Ser  
1 5 10 15

Ile Gly Gly Ala Gly Gly Asn Asn Gly Leu Leu Gly Thr Ser Arg Gln  
20 25 30

35 Asn Ala Gly Leu Gly Gly Asn Ser Ala Leu Gly Leu Gly Gly Gly Asn  
35 40 45

Gln Asn Asp Thr Val Asn Gln Leu Ala Gly Leu Leu Thr Gly Met Met  
50 55 60

Met Met Met Ser Met Met Gly Gly Gly Gly Leu Met Gly Gly Gly Leu  
65 70 75 80

45 Gly Gly Gly Leu Gly Asn Gly Leu Gly Gly Ser Gly Gly Leu Gly Glu  
85 90 95

Gly Leu Ser Asn Ala Leu Asn Asp Met Leu Gly Gly Ser Leu Asn Thr  
100 105 110

50

- 16 -

	Leu	Gly	Ser	Lys	Gly	Gly	Asn	Asn	Thr	Thr	Ser	Thr	Thr	Asn	Ser	Pro
			115					120					125			
5	Leu	Asp	Gln	Ala	Leu	Gly	Ile	Asn	Ser	Thr	Ser	Gln	Asn	Asp	Asp	Ser
		130					135					140				
	Thr	Ser	Gly	Thr	Asp	Ser	Thr	Ser	Asp	Ser	Ser	Asp	Pro	Met	Gln	Gln
	145				150						155					160
10	Leu	Leu	Lys	Met	Phe	Ser	Glu	Ile	Met	Gln	Ser	Leu	Phe	Gly	Asp	Gly
					165					170					175	
	Gln	Asp	Gly	Thr	Gln	Gly	Ser	Ser	Ser	Gly	Gly	Lys	Gln	Pro	Thr	Glu
				180					185					190		
15	Gly	Glu	Gln	Asn	Ala	Tyr	Lys	Lys	Gly	Val	Thr	Asp	Ala	Leu	Ser	Gly
			195					200					205			
20	Leu	Met	Gly	Asn	Gly	Leu	Ser	Gln	Leu	Leu	Gly	Asn	Gly	Gly	Leu	Gly
		210					215					220				
	Gly	Gly	Gln	Gly	Gly	Asn	Ala	Gly	Thr	Gly	Leu	Asp	Gly	Ser	Ser	Leu
	225					230					235					240
25	Gly	Gly	Lys	Gly	Leu	Gln	Asn	Leu	Ser	Gly	Pro	Val	Asp	Tyr	Gln	Gln
					245					250					255	
	Leu	Gly	Asn	Ala	Val	Gly	Thr	Gly	Ile	Gly	Met	Lys	Ala	Gly	Ile	Gln
				260					265					270		
30	Ala	Leu	Asn	Asp	Ile	Gly	Thr	His	Arg	His	Ser	Ser	Thr	Arg	Ser	Phe
			275					280					285			
35	Val	Asn	Lys	Gly	Asp	Arg	Ala	Met	Ala	Lys	Glu	Ile	Gly	Gln	Phe	Met
		290					295					300				
	Asp	Gln	Tyr	Pro	Glu	Val	Phe	Gly	Lys	Pro	Gln	Tyr	Gln	Lys	Gly	Pro
	305					310					315					320
40	Gly	Gln	Glu	Val	Lys	Thr	Asp	Asp	Lys	Ser	Trp	Ala	Lys	Ala	Leu	Ser
					325					330					335	
	Lys	Pro	Asp	Asp	Asp	Gly	Met	Thr	Pro	Ala	Ser	Met	Glu	Gln	Phe	Asn
				340					345					350		
45	Lys	Ala	Lys	Gly	Met	Ile	Lys	Arg	Pro	Met	Ala	Gly	Asp	Thr	Gly	Asn
			355					360					365			
50	Gly	Asn	Leu	Gln	Ala	Arg	Gly	Ala	Gly	Gly	Ser	Ser	Leu	Gly	Ile	Asp
		370					375					380				
	Ala	Met	Met	Ala	Gly	Asp	Ala	Ile	Asn	Asn	Met	Ala	Leu	Gly	Lys	Leu
	385					390					395					400
55	Gly	Ala	Ala													

This hypersensitive response elicitor polypeptide or  
 60 protein has a molecular weight of about 39 kDa, has a pI  
 of approximately 4.3, and is heat stable at 100°C for at

- 17 -

least 10 minutes. This hypersensitive response elicitor polypeptide or protein has substantially no cysteine. The hypersensitive response elicitor polypeptide or protein derived from *Erwinia amylovora* is more fully described in Wei, Z.-M., R. J. Laby, C. H. Zumoff, D. W. Bauer, S.-Y. He, A. Collmer, and S. V. Beer, "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which is hereby incorporated by reference. The DNA molecule encoding this polypeptide or protein has a nucleotide sequence corresponding to SEQ. ID. No. 4 as follows:

15	AAGCTTCGGC ATGGCACGTT TGACCGTTGG GTCGGCAGGG TACGTTTGAA TTATTCATAA	60
	GAGGAATACG TTATGAGTCT GAATACAAGT GGGCTGGGAG CGTCAACGAT GCAAATTTCT	120
	ATCGGCGGTG CGGGCGGAAA TAACGGGTTG CTGGGTACCA GTCGCCAGAA TGCTGGGTTG	180
20	GGTGGCAATT CTGCACTGGG GCTGGGCGGC GGTAATCAAA ATGATACCGT CAATCAGCTG	240
	GCTGGCTTAC TCACCGGCAT GATGATGATG ATGAGCATGA TGGGCGGTGG TGGGCTGATG	300
25	GGCGGTGGCT TAGGCGGTGG CTTAGGTAAT GGCTTGGGTG GCTCAGGTGG CCTGGGCGAA	360
	GGACTGTGCGA ACGCGCTGAA CGATATGTGA GGCGGTTCGC TGAACACGCT GGGCTCGAAA	420
	GGCGGCAACA ATACCACTTC AACAACAAAT TCCCCGCTGG ACCAGGCGCT GGGTATTAAC	480
30	TCAACGTCCC AAAACGACGA TTCCACCTCC GGCACAGATT CCACCTCAGA CTCCAGCGAC	540
	CCGATGCAGC AGCTGCTGAA GATGTTTCAGC GAGATAATGC AAAGCCTGTT TGGTGATGGG	600
35	CAAGATGGCA CCCAGGGCAG TTCCTCTGGG GGCAAGCAGC CGACCGAAGG CGAGCAGAAC	660
	GCCTATAAAA AAGGAGTCAC TGATGCGCTG TCGGGCCTGA TGGGTAATGG TCTGAGCCAG	720
	CTCCTTGGCA ACGGGGGACT GGGAGGTGGT CAGGGCGGTA ATGCTGGCAC GGGTCTTGAC	780
40	GGTTCGTCGC TGGGCGGCAA AGGGCTGCAA AACCTGAGCG GGCCGGTGA CTACCAGCAG	840
	TTAGGTAACG CCGTGGGTAC CGGTATCGGT ATGAAAGCGG GCATTGAGG GCTGAATGAT	900
45	ATCGGTACGC ACAGGCACAG TTCAACCCGT TCTTTCGTCA ATAAAGGCGA TCGGGCGATG	960
	GCGAAGGAAA TCGGTCAGTT CATGGACCAG TATCCTGAGG TGTTTGGCAA GCCGCAGTAC	1020
	CAGAAAGGCC CGGGTCAGGA GGTGAAAACC GATGACAAAT CATGGGCAA AGCACTGAGC	1080
50	AAGCCAGATG ACGACGGAAT GACACCAGCC AGTATGGAGC AGTTCAACAA AGCCAAGGGC	1140
	ATGATCAAAA GGCCCATGGC GGGTGATACC GGCAACGGCA ACCTGCAGGC ACGCGGTGCC	1200
55	GGTGGTTCTT CGCTGGGTAT TGATGCCATG ATGGCCGGTG ATGCCATTAA CAATATGGCA	1260
	CTTGGCAAGC TGGGCGCGGC TTAAGCTT	1288

- 18 -

The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID. No. 5 as follows:

5	Met	Gln	Ser	Leu	Ser	Leu	Asn	Ser	Ser	Ser	Leu	Gln	Thr	Pro	Ala	Met	1	5	10	15
10	Ala	Leu	Val	Leu	Val	Arg	Pro	Glu	Ala	Glu	Thr	Thr	Gly	Ser	Thr	Ser	20	25	30	
	Ser	Lys	Ala	Leu	Gln	Glu	Val	Val	Val	Lys	Leu	Ala	Glu	Glu	Leu	Met	35	40	45	
15	Arg	Asn	Gly	Gln	Leu	Asp	Asp	Ser	Ser	Pro	Leu	Gly	Lys	Leu	Leu	Ala	50	55	60	
20	Lys	Ser	Met	Ala	Ala	Asp	Gly	Lys	Ala	Gly	Gly	Gly	Ile	Glu	Asp	Val	65	70	75	80
	Ile	Ala	Ala	Leu	Asp	Lys	Leu	Ile	His	Glu	Lys	Leu	Gly	Asp	Asn	Phe	85	90	95	
25	Gly	Ala	Ser	Ala	Asp	Ser	Ala	Ser	Gly	Thr	Gly	Gln	Gln	Asp	Leu	Met	100	105	110	
	Thr	Gln	Val	Leu	Asn	Gly	Leu	Ala	Lys	Ser	Met	Leu	Asp	Asp	Leu	Leu	115	120	125	
30	Thr	Lys	Gln	Asp	Gly	Gly	Thr	Ser	Phe	Ser	Glu	Asp	Asp	Met	Pro	Met	130	135	140	
35	Leu	Asn	Lys	Ile	Ala	Gln	Phe	Met	Asp	Asp	Asn	Pro	Ala	Gln	Phe	Pro	145	150	155	160
	Lys	Pro	Asp	Ser	Gly	Ser	Trp	Val	Asn	Glu	Leu	Lys	Glu	Asp	Asn	Phe	165	170	175	
40	Leu	Asp	Gly	Asp	Glu	Thr	Ala	Ala	Phe	Arg	Ser	Ala	Leu	Asp	Ile	Ile	180	185	190	
	Gly	Gln	Gln	Leu	Gly	Asn	Gln	Gln	Ser	Asp	Ala	Gly	Ser	Leu	Ala	Gly	195	200	205	
45	Thr	Gly	Gly	Gly	Leu	Gly	Thr	Pro	Ser	Ser	Phe	Ser	Asn	Asn	Ser	Ser	210	215	220	
50	Val	Met	Gly	Asp	Pro	Leu	Ile	Asp	Ala	Asn	Thr	Gly	Pro	Gly	Asp	Ser	225	230	235	240
	Gly	Asn	Thr	Arg	Gly	Glu	Ala	Gly	Gln	Leu	Ile	Gly	Glu	Leu	Ile	Asp	245	250	255	
55	Arg	Gly	Leu	Gln	Ser	Val	Leu	Ala	Gly	Gly	Gly	Leu	Gly	Thr	Pro	Val	260	265	270	
	Asn	Thr	Pro	Gln	Thr	Gly	Thr	Ser	Ala	Asn	Gly	Gly	Gln	Ser	Ala	Gln	275	280	285	

- 19 -

Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Leu Lys Gly Leu Glu Ala  
 290 295 300  
 5 Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala  
 305 310 315 320  
 Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg  
 325 330 335  
 10 Asn Gln Ala Ala Ala  
 340

This hypersensitive response elicitor polypeptide or  
 15 protein has a molecular weight of 34-35 kDa. It is rich  
 in glycine (about 13.5%) and lacks cysteine and tyrosine.  
 Further information about the hypersensitive response  
 elicitor derived from *Pseudomonas syringae* is found in  
 He, S. Y., H. C. Huang, and A. Collmer, "Pseudomonas  
 20 *syringae* pv. *syringae* Harpin<sub>PSS</sub>: a Protein that is  
 Secreted via the Hrp Pathway and Elicits the  
 Hypersensitive Response in Plants," Cell 73:1255-1266  
 (1993), which is hereby incorporated by reference. The  
 DNA molecule encoding the hypersensitive response  
 25 elicitor from *Pseudomonas syringae* has a nucleotide  
 sequence corresponding to SEQ. ID. No. 6 as follows:

ATGCAGAGTC TCAGTCTTAA CAGCAGCTCG CTGCAAACCC CGGCAATGGC CCTTGTCTCTG 60  
 30 GTACGTCTCTG AAGCCGAGAC GACTGGCAGT ACGTCGAGCA AGGCGCTTCA GGAAGTTGTC 120  
 GTGAAGCTGG CCGAGGAACT GATGCGCAAT GGTCAACTCG ACGACAGCTC GCCATTGGGA 180  
 AACTGTTGG CCAAGTCGAT GGCCGCAGAT GGCAAGGCGG GCGGCGGTAT TGAGGATGTC 240  
 35 ATCGCTGCGC TGGACAAGCT GATCCATGAA AAGCTCGGTG ACAACTTCGG CGCGTCTGCG 300  
 GACAGCGCCT CGGGTACCGG ACAGCAGGAC CTGATGACTC AGGTGCTCAA TGGCCTGGCC 360  
 40 AAGTCGATGC TCGATGATCT TCTGACCAAG CAGGATGGCG GGACAAGCTT CTCCGAAGAC 420  
 GATATGCCGA TGCTGAACAA GATCGCGCAG TTCATGGATG ACAATCCCGC ACAGTTTCCC 480  
 AAGCCGGACT CGGGCTCTG GGTGAACGAA CTCAAGGAAG ACAACTTCCT TGATGGCGAC 540  
 45 GAAACGGCTG CGTTCCGTTC GGCACTCGAC ATCATTGGCC AGCAACTGGG TAATCAGCAG 600  
 AGTGACGCTG GCAGTCTGGC AGGGACGGGT GGAGGTCTGG GCACTCCGAG CAGTTTTTCC 660  
 50 AACAACTCGT CCGTGATGGG TGATCCGCTG ATCGACGCCA ATACCGGTCC CGGTGACAGC 720  
 GGCAATACCC GTGGTGAAGC GGGGCAACTG ATCGGCGAGC TTATCGACCG TGGCCTGCAA 780  
 TCGGTATTGG CCGGTGGTGG ACTGGGCACA CCCGTAAACA CCCCAGAC CGGTACGTGC 840

- 20 -

GCGAATGGCG GACAGTCCGC TCAGGATCTT GATCAGTTGC TGGGCGGCTT GCTGCTCAAG 900  
 GGCTGGAGG CAACGCTCAA GGATGCCGGG CAAACAGGCA CCGACGTGCA GTCGAGCGCT 960  
 5 GCGCAAATCG CCACCTTGCT GGTCAGTACG CTGCTGCAAG GCACCCGCAA TCAGGCTGCA 1020  
 GCCTGA 1026

10 The hypersensitive response elicitor  
 polypeptide or protein derived from *Pseudomonas*  
*solanacearum* has an amino acid sequence corresponding to  
 SEQ. ID. No. 7 as follows:

15 Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln  
 1 5 10 15  
 Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser  
 20 20 25 30  
 Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile  
 35 40 45  
 25 Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly  
 50 55 60  
 Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala  
 65 70 75 80  
 30 Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser  
 85 90 95  
 Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met  
 100 105 110  
 35 Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala  
 115 120 125  
 40 Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val  
 130 135 140  
 Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala  
 145 150 155 160  
 45 Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly Gly  
 165 170 175  
 Gly Ala Gly Ala Gly Gly Ala Gly Gly Gly Val Gly Gly Ala Gly Gly  
 180 185 190  
 50 Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Gly Gly Ala Asn Gly Ala  
 195 200 205  
 55 Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn  
 210 215 220  
 Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp  
 225 230 235 240  
 60

- 21 -

	Gln Gly Gly Leu Thr Gly Val Leu Gln Lys Leu Met Lys Ile Leu Asn	245	250	255
5	Ala Leu Val Gln Met Met Gln Gln Gly Gly Leu Gly Gly Gly Asn Gln	260	265	270
	Ala Gln Gly Gly Ser Lys Gly Ala Gly Asn Ala Ser Pro Ala Ser Gly	275	280	285
10	Ala Asn Pro Gly Ala Asn Gln Pro Gly Ser Ala Asp Asp Gln Ser Ser	290	295	300
	Gly Gln Asn Asn Leu Gln Ser Gln Ile Met Asp Val Val Lys Glu Val	305	310	315
15	Val Gln Ile Leu Gln Gln Met Leu Ala Ala Gln Asn Gly Gly Ser Gln	325	330	335
20	Gln Ser Thr Ser Thr Gln Pro Met	340		

It is encoded by a DNA molecule having a nucleotide sequence corresponding SEQ. ID. No. 8 as follows:

25	ATGTCAGTCG GAAACATCCA GAGCCCGTCG AACCTCCCGG GTCTGCAGAA CCTGAACCTC	60
	AACACCAACA CCAACAGCCA GCAATCGGGC CAGTCCGTGC AAGACCTGAT CAAGCAGGTC	120
30	GAGAAGGACA TCCTCAACAT CATCGCAGCC CTCGTGCAGA AGGCCGCACA GTCGGCGGGC	180
	GGCAACACCG GTAACACCGG CAACGCGCCG GCGAAGGACG GCAATGCCAA CGCGGGCGCC	240
	AACGACCCGA GCAAGAACGA CCCGAGCAAG AGCCAGGCTC CGCAGTCGGC CAACAAGACC	300
35	GGCAACGTCG ACGACGCCAA CAACCAGGAT CCGATGCAAG CGCTGATGCA GCTGCTGGAA	360
	GACCTGGTGA AGCTGCTGAA GGC GGCCCTG CACATGCAGC AGCCCGGCGG CAATGACAAG	420
40	GGCAACGGCG TGGGCGGTGC CAACGCGGCC AAGGGTGCCG GCGGCCAGGG CGGCCTGGCC	480
	GAAGCGCTGC AGGAGATCGA GCAGATCCTC GCCCAGCTCG GCGGCGGCGG TGCTGGCGCC	540
	GGCGGCGCGG GTGGCGGTGT CGGCGGTGCT GGTGGCGCGG ATGGCGGCTC CGGTGCGGGT	600
45	GGCGCAGGCG GTGCGAACGG CGCCGACGGC GGCAATGGCG TGAACGGCAA CCAGGCGAAC	660
	GGCCCCGAGA ACGCAGGCGA TGTCAACGGT GCCAACGGCG CGGATGACGG CAGCGAAGAC	720
50	CAGGGCGGCC TCACCGGCGT GCTGCAAAAG CTGATGAAGA TCCTGAACGC GCTGGTGCAG	780
	ATGATGCAGC AAGGCGGCCT CGGCGGCGGC AACCAGGCGC AGGGCGGCTC GAAGGGTGCC	840
	GGCAACGCCT CGCCGGCTTC CGGCGCGAAC CCGGGCGCGA ACCAGCCCGG TTCGGCGGAT	900
55	GATCAATCGT CCGGCCAGAA CAATCTGCAA TCCCAGATCA TGGATGTGGT GAAGGAGGTC	960
	GTCCAGATCC TGCAGCAGAT GCTGGCGGCG CAGAACGGCG GCAGCCAGCA GTCCACCTCG	1020
60	ACGCAGCCGA TGTA	1035

- 22 -

Further information regarding the hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* is set forth in Arlat, M., F. Van Gijsegem, J. C. Huet, J. C. Pemollet, and C. A. Boucher, "PopA1, a  
 5 Protein which Induces a Hypersensitive-like Response in Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-533 (1994), which is hereby incorporated by reference.

The hypersensitive response elicitor  
 10 polypeptide or protein from *Xanthomonas campestris* pv. glycines has an amino acid sequence corresponding to SEQ. ID. No. 9 as follows:

```

15      Thr Leu Ile Glu Leu Met Ile Val Val Ala Ile Ile Ala Ile Leu Ala
        1           5           10           15
      Ala Ile Ala Leu Pro Ala Tyr Gln Asp Tyr
                20           25
  
```

20 This sequence is an amino terminal sequence having 26 residues only from the hypersensitive response elicitor polypeptide or protein of *Xanthomonas campestris* pv. glycines. It matches with fimbrial subunit proteins  
 25 determined in other *Xanthomonas campestris* pathovars.

The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* pv. *pelargonii* is heat stable, protease sensitive, and has a molecular weight of 20 kDa. It includes an amino acid  
 30 sequence corresponding to SEQ. ID. No. 10 as follows:

```

35      Ser Ser Gln Gln Ser Pro Ser Ala Gly Ser Glu Gln Gln Leu Asp Gln
        1           5           10           15
      Leu Leu Ala Met
                20
  
```

40 Isolation of *Erwinia carotovora* hypersensitive response elicitor protein or polypeptide is described in Cui et al., "The RsmA Mutants of *Erwinia carotovora*



- 23 -

subsp. *carotovora* Strain Ecc71 Overexpress *hrp* N<sub>Ecc</sub> and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI, 9(7):565-73 (1996), which is hereby incorporated by reference. The hypersensitive response elicitor protein or polypeptide is shown in Ahmad et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microbe Interact., July 14-19, 1996 and Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc., July 27-31, 1996, which are hereby incorporated by reference.

Hypersensitive response elicitor proteins or polypeptides from *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamoni*, *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora* are described in Kaman, et al., "Extracellular Protein Elicitors from *Phytophthora*: Most Specificity and Induction of Resistance to Bacterial and Fungal Phytopathogens," Molec. Plant-Microbe Interact., 6(1):15-25 (1993), Ricci et al., "Structure and Activity of Proteins from Pathogenic Fungi *Phytophthora* Eliciting Necrosis and Acquired Resistance in Tobacco," Eur. J. Biochem., 183:555-63 (1989), Ricci et al., "Differential Production of Parasiticein, and Elicitor of Necrosis and Resistance in Tobacco, by Isolates of *Phytophthora parasitica*," Plant Path. 41:298-307 (1992), Baillreul et al, "A New Elicitor of the Hypersensitive Response in Tobacco: A Fungal Glycoprotein Elicits Cell Death, Expression of Defence Genes, Production of Salicylic Acid, and Induction of Systemic Acquired Resistance," Plant J., 8(4):551-60 (1995), and Bonnet et al., "Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants," Eur. J. Plant Path., 102:181-92 (1996), which are hereby incorporated by reference.

- 24 -

The above elicitors are exemplary. Other elicitors can be identified by growing fungi or bacteria that elicit a hypersensitive response under which genes encoding an elicitor are expressed. Cell-free  
5 preparations from culture supernatants can be tested for elicitor activity (i.e. local necrosis) by using them to infiltrate appropriate plant tissues.

It is also possible to use fragments of the above hypersensitive response elicitor polypeptides or  
10 proteins as well as fragments of full length elicitors from other pathogens, in the method of the present invention.

Suitable fragments can be produced by several means. In the first, subclones of the gene encoding a known elicitor protein are produced by conventional molecular genetic manipulation by subcloning gene  
5 fragments. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or a peptide that can be tested for elicitor activity according to the procedure described below.

As an alternative, fragments of an elicitor  
10 protein can be produced by digestion of a full-length elicitor protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave elicitor proteins at different sites based on the amino  
15 acid sequence of the elicitor protein. Some of the fragments that result from proteolysis may be active elicitors of resistance.

In another approach, based on knowledge of the primary structure of the protein, fragments of the  
20 elicitor protein gene may be synthesized by using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. These then would be cloned into an appropriate vector for

- 25 -

increase and expression of a truncated peptide or protein.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences for the elicitor being produced. Alternatively, subjecting a full length elicitor to high temperatures and pressures will produce fragments. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE).

10 An example of a useful fragment is the popA1 fragment of the hypersensitive response elicitor polypeptide or protein from *Pseudomonas solanacearum*. See Arlat, M., F. Van Gijsegem, J.C. Huet, J.C. Pemollet, and C.A. Boucher, "PopA1, a Protein Which Induces a  
15 Hypersensitive-like Response in Specific Petunia Genotypes is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-53 (1994), which is hereby incorporated by reference. As to *Erwinia amylovora*, a suitable fragment can be, for example, either or both the  
20 polypeptide extending between and including amino acids 1 and 98 of SEQ. ID. No. 3 and the polypeptide extending between and including amino acids 137 and 204 of SEQ. ID. No. 3.

Variants may also (or alternatively) be  
25 modified by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure and hydrophobic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-  
30 terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide.

- 26 -

The protein or polypeptide of the present invention is preferably produced in purified form (preferably at least about 60%, more preferably 80%, pure) by conventional techniques. Typically, the protein or polypeptide of the present invention is produced but not secreted into the growth medium of recombinant host cells. Alternatively, the protein or polypeptide of the present invention is secreted into growth medium. In the case of unsecreted protein, to isolate the protein, the host cell (e.g., *E. coli*) carrying a recombinant plasmid is propagated, lysed by sonication, heat, or chemical treatment, and the homogenate is centrifuged to remove bacterial debris. The supernatant is then subjected to heat treatment and the hypersensitive response elicitor protein is separated by centrifugation. The supernatant fraction containing the polypeptide or protein of the present invention is subjected to gel filtration in an appropriately sized dextran or polyacrylamide column to separate the proteins. If necessary, the protein fraction may be further purified by ion exchange or HPLC.

The DNA molecule encoding the hypersensitive response elicitor polypeptide or protein can be incorporated in cells using conventional recombinant DNA technology. Generally, this involves inserting the DNA molecule into an expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in proper sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage

- 27 -

and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including procaryotic organisms and eucaryotic cells grown in tissue culture.

5           Recombinant genes may also be introduced into viruses, such as vaccina virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

          Suitable vectors include, but are not limited  
10 to, the following viral vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning  
15 Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which is hereby incorporated by reference), pQE, pIH821, pGEX, pET series (see F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression Technology vol. 185 (1990), which is  
20 hereby incorporated by reference), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard  
25 cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference.

          A variety of host-vector systems may be  
30 utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA;  
35 microorganisms such as yeast containing yeast vectors;

- 28 -

mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression elements of these  
5 vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

Different genetic signals and processing events  
10 control many levels of gene expression (e.g., DNA transcription and messenger RNA (mRNA) translation).

Transcription of DNA is dependent upon the presence of a promotor which is a DNA sequence that directs the binding of RNA polymerase and thereby  
15 promotes mRNA synthesis. The DNA sequences of eucaryotic promoters differ from those of procaryotic promoters. Furthermore, eucaryotic promoters and accompanying genetic signals may not be recognized in or may not function in a procaryotic system, and, further,  
20 procaryotic promoters are not recognized and do not function in eucaryotic cells.

Similarly, translation of mRNA in procaryotes depends upon the presence of the proper procaryotic signals which differ from those of eucaryotes. Efficient  
25 translation of mRNA in procaryotes requires a ribosome binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG, which encodes the amino-terminal methionine of the  
30 protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct positioning of the ribosome. For a review on maximizing gene expression, see Roberts and

- 29 -

Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference.

Promoters vary in their "strength" (i.e. their ability to promote transcription). For the purposes of  
5 expressing a cloned gene, it is desirable to use strong promoters in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a number of suitable promoters may be used. For  
10 instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promoters such as the T7 phage promoter, *lac* promoter, *trp* promoter, *recA* promoter, ribosomal RNA promoter, the  $P_R$  and  $P_L$  promoters of coliphage lambda and others, including but not limited, to *lacUV5*, *ompF*, *bla*,  
15 *lpp*, and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lacUV5* (*tac*) promoter or other *E. coli* promoters produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription  
20 of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promoter unless specifically induced. In certain operations, the addition of specific inducers is  
25 necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

30 Specific initiation signals are also required for efficient gene transcription and translation in procaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein  
35 synthesized, respectively. The DNA expression vector,

- 30 -

which contains a promotor, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires an SD sequence about 7-9 bases 5' to the initiation codon (ATG) to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan *E*, *D*, *C*, *B* or *A* genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

Once the isolated DNA molecule encoding the hypersensitive response elicitor polypeptide or protein has been cloned into an expression system, it is ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like.

The method of the present invention can be utilized to treat a wide variety of plants or their seeds to enhance growth. Suitable plants include dicots and monocots. More particularly, useful crop plants can include: rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane. Examples of suitable ornamental plants are: rose, *Saintpaulia*,



- 31 -

petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

The method of the present invention involving application of the hypersensitive response elicitor polypeptide or protein can be carried out through a variety of procedures when all or part of the plant is treated, including leaves, stems, roots, etc. This may (but need not) involve infiltration of the hypersensitive response elicitor polypeptide or protein into the plant. Suitable application methods include topical application (e.g., high or low pressure spraying), injection, dusting, and leaf abrasion proximate to when elicitor application takes place. When treating plant seeds, in accordance with the application embodiment of the present invention, the hypersensitive response elicitor protein or polypeptide can be applied by topical application (low or high pressure spraying), coating, immersion, dusting, or injection. Other suitable application procedures can be envisioned by those skilled in the art provided they are able to effect contact of the hypersensitive response elicitor polypeptide or protein with cells of the plant or plant seed. Once treated with the hypersensitive response elicitor of the present invention, the seeds can be planted in natural or artificial soil and cultivated using conventional procedures to produce plants. After plants have been propagated from seeds treated in accordance with the present invention, the plants may be treated with one or more applications of the hypersensitive response elicitor protein or polypeptide to enhance growth in the plants. Such propagated plants may, in turn, be useful in producing seeds or propagules (e.g., cuttings) that produce plants capable of enhanced growth.

The hypersensitive response elicitor polypeptide or protein can be applied to plants or plant

- 32 -

seeds in accordance with the present invention alone or in a mixture with other materials. Alternatively, the hypersensitive response elicitor polypeptide or protein can be applied separately to plants with other materials  
5 being applied at different times.

A composition suitable for treating plants or plant seeds in accordance with the application embodiment of the present invention contains a hypersensitive response elicitor polypeptide or protein in a carrier.  
10 Suitable carriers include water, aqueous solutions, slurries, or dry powders. In this embodiment, the composition contains greater than 0.5 nM hypersensitive response elicitor polypeptide or protein.

Although not required, this composition may  
15 contain additional additives including fertilizer, insecticide, fungicide, nematocide, herbicide, and mixtures thereof. Suitable fertilizers include  $(\text{NH}_4)_2\text{NO}_3$ . An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

20 Other suitable additives include buffering agents, wetting agents, coating agents, and abrading agents. These materials can be used to facilitate the process of the present invention. In addition, the hypersensitive response elicitor polypeptide or protein  
25 can be applied to plant seeds with other conventional seed formulation and treatment materials, including clays and polysaccharides.

In the alternative embodiment of the present invention involving the use of transgenic plants and  
30 transgenic seeds, a hypersensitive response elicitor polypeptide or protein need not be applied topically to the plants or seeds. Instead, transgenic plants transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein are produced  
35 according to procedures well known in the art, such as by

- 33 -

biolistics or *Agrobacterium* mediated transformation. Examples of suitable hypersensitive response elicitor polypeptides or proteins and the nucleic acid sequences for their encoding DNA are disclosed *supra*. Once  
5 transgenic plants of this type are produced, the plants themselves can be cultivated in accordance with conventional procedure with the presence of the gene encoding the hypersensitive response elicitor resulting in enhanced growth of the plant. Alternatively,  
10 transgenic seeds are recovered from the transgenic plants. These seeds can then be planted in the soil and cultivated using conventional procedures to produce transgenic plants. The transgenic plants are propagated from the planted transgenic seeds under conditions  
15 effective to impart enhanced growth. While not wishing to be bound by theory, such growth enhancement may be RNA mediated or may result from expression of the elicitor polypeptide or protein.

When transgenic plants and plant seeds are used  
20 in accordance with the present invention, they additionally can be treated with the same materials as are used to treat the plants and seeds to which a hypersensitive response elicitor polypeptide or protein is applied. These other materials, including  
25 hypersensitive response elicitors, can be applied to the transgenic plants and plant seeds by the above-noted procedures, including high or low pressure spraying, injection, coating, dusting, and immersion. Similarly, after plants have been propagated from the transgenic  
30 plant seeds, the plants may be treated with one or more applications of the hypersensitive response elicitor to enhance plant growth. Such plants may also be treated with conventional plant treatment agents (e.g., insecticides, fertilizers, etc.). The transgenic plants  
35 of the present invention are useful in producing seeds or

- 34 -

propagules (e.g., cuttings) from which plants capable of enhanced growth would be produced.

#### EXAMPLES

5

Example 1 - Effect of Treating Tomato Seeds with *Erwinia amylovora* Hypersensitive Response Elicitor on Germination Percentage

10

Seeds of the Marglobe Tomato Variety were submerged in 40ml of *Erwinia amylovora* hypersensitive response elicitor solution ("harpin"). Harpin was prepared by growing *E. coli* strain DH5 containing the plasmid pCPP2139 (see Figure 1), lysing the cells by sonication, heat treating by holding in boiling water for 15 5 minutes before centrifuging to remove cellular debris, and precipitating proteins and other heat-labile components. The resulting preparation ("CFEP") was diluted serially. These dilutions (1:40, 1:80, 1:160, 20 1:320 and 1:640) contained 20, 10, 5, 2.5, and 1.25  $\mu\text{gm/ml}$ , respectively, of harpin based on Western Blot assay. Seeds were soaked in harpin or buffer in beakers on day 0 for 24 hours at 28°C in a growth chamber. After soaking, the seeds were sown in germination pots with 25 artificial soil on day 1. This procedure was carried out on 100 seeds per treatment.

#### **Treatments:**

30

1. Seeds in harpin (1:40) (20  $\mu\text{gm/ml}$ ).
2. Seeds in harpin (1:80) (10  $\mu\text{gm/ml}$ ).
3. Seeds in harpin (1:160) (5  $\mu\text{gm/ml}$ ).
4. Seeds in harpin (1:320) (2.5  $\mu\text{gm/ml}$ ).
5. Seeds in harpin (1:640) (1.25  $\mu\text{gm/ml}$ ).
6. Seeds in buffer (5mM  $\text{KPO}_4$ , pH 6.8).

35

- 35 -

Table 1 - Number of Seedlings After Seed Treatment

Treatment	Number of seeds germinated				
	Day 0	Day 1	Day 5	Day 7	Day 9
5 Harpin seed soak (20 $\mu$ gm/ml)	sowing	43	57	59	
Harpin seed soak (10 $\mu$ gm/ml)	sowing	43	52	52	
Harpin seed soak (5 $\mu$ gm/ml)	sowing	40	47	51	
Harpin seed soak (2.5 $\mu$ gm/ml)	sowing	43	56	58	
10 Harpin seed soak (1.25 $\mu$ gm/ml)	sowing	38	53	57	
Buffer seed soak	sowing	27	37	40	

As shown in Table 1, the treatment of tomato seeds with *Erwinia amylovora* hypersensitive response elicitor reduced the time needed for germination and greatly increased the percentage of germination.

#### Example 2 - Effect of Treating Tomato Seeds with *Erwinia amylovora* Hypersensitive Response Elicitor on Tomato Plant Height

Seeds of the Marglobe Tomato Variety were submerged in *Erwinia amylovora* harpin (1:15, 1:30, 1:60, and 1:120) or buffer in beakers on day 0 for 24 hours at 28°C in a growth chamber. After soaking, the seeds were sown in germination pots with artificial soil on day 1.

Ten uniform appearing plants per treatment were chosen randomly and measured. The seedlings were measured by ruler from the surface of soil to the top of plant.

#### Treatments:

1. Harpin (1:15) (52  $\mu$ gm/ml).
2. Harpin (1:30) (26  $\mu$ gm/ml).
3. Harpin (1:60) (13  $\mu$ gm/ml).
4. Harpin (1:120) (6.5  $\mu$ gm/ml).
5. Buffer (5mM KPO<sub>4</sub>, pH 6.8).

Table 2 - Seedling Height (cm) 15 Days After Seed Treatment.

Treat	Plants	1	2	3	4	5	6	7	8	9	10	Mean
52 $\mu$ gm/ml	10	5.6	5.8	5.8	5.6	6.0	6.0	5.8	5.4	5.8	5.6	5.7
26 $\mu$ gm/ml	10	6.8	7.2	6.6	7.0	6.8	6.8	7.0	7.4	7.2	7.0	7.0
13 $\mu$ gm/ml	10	5.8	5.6	6.0	5.6	5.8	5.8	5.6	5.8	6.0	5.6	5.9
6.5 $\mu$ gm/ml	10	5.4	5.2	5.6	5.4	5.2	5.4	5.6	5.6	5.4	5.2	5.4
Buffer	10	5.6	5.4	5.2	5.2	5.4	5.2	5.0	5.2	5.4	5.6	5.3

Table 3 - Seedling Height (cm) 21 Days After Seed Treatment.

Treat	Plants	1	2	3	4	5	6	7	8	9	10	Mean
52 $\mu$ gm/ml	10	7.6	7.8	7.6	7.6	7.8	7.8	7.8	7.4	7.6	7.6	7.7
26 $\mu$ gm/ml	10	8.2	8.2	8.0	9.0	8.4	8.6	8.6	9.0	9.2	9.0	8.6
13 $\mu$ gm/ml	10	6.8	6.6	6.8	6.8	6.8	6.8	6.6	7.2	7.0	7.2	6.9
6.5 $\mu$ gm/ml	10	6.8	6.6	6.6	6.4	6.8	6.6	6.8	6.6	6.6	6.8	6.7
Buffer	10	6.6	6.4	6.2	6.6	6.4	6.6	6.8	6.4	6.4	6.6	6.5

Table 4 - Seedling Height (cm) 27 Days After Seed Treatment.

Treat	1	2	3	4	5	6	7	8	9	10	Mean
52 $\mu$ gm/ml	10.2	10.6	10.4	10.6	10.4	10.6	10.8	10.4	10.8	10.6	10.5
26 $\mu$ gm/ml	11.6	11.4	11.6	11.8	11.8	11.8	11.6	11.4	11.6	11.4	11.6
13 $\mu$ gm/ml	9.8	9.6	9.8	9.6	9.8	9.8	9.6	9.4	9.6	9.8	9.7
6.5 $\mu$ gm/ml	9.4	9.4	9.6	9.4	9.6	9.4	9.6	9.6	9.4	9.2	9.5
Buffer	9.6	10.2	10.0	9.8	10.0	10.2	10.0	10.2	10.4	9.6	10.0

- 38 -

Table 5 - Summary--Mean Height of Tomato Plants after Treatment.

5	Treatment	Mean height of tomato plants(cm)				
	Day 0	Day 1	Day 15	Day 21	Day 27	
	Harpin seed soak (1:15)	sowing	5.7	7.7	10.5	
	Harpin seed soak (1:30)	sowing	7.0	8.6	11.6	
	Harpin seed soak (1:60)	sowing	5.9	6.9	9.7	
10	Harpin seed soak (1:120)	sowing	5.4	6.7	9.5	
	Buffer seed soak	sowing	5.3	6.5	10.0	

As shown in Tables 2-5, the treatment of tomato seeds with *Erwinia amylovora* hypersensitive response elicitor increased plant growth. A 1:30 dilution had the greatest effect -- a 16% increase in seedling height.

#### Example 3 - Effect of Treating Tomato Plants with *Erwinia amylovora* Hypersensitive Response Elicitor on Tomato Plant Height

When Marglobe tomato plants were 4 weeks old, they were sprayed with 6 ml/plant of *Erwinia amylovora* harpin solution containing 13  $\mu\text{gm/ml}$  (1:60) or 8.7  $\mu\text{gm/ml}$  (1:90) of harpin or buffer (5mM  $\text{KPO}_4$ ) in a growth chamber at 28°C. The heights of tomato plants were measured 2 weeks after spraying harpin (6-week-old tomato plants) and 2 weeks plus 5 days after spraying. Ten uniform appearing plants per treatment were chosen randomly and measured. The seedlings were measured by ruler from the surface of soil to the top of plant.

#### Treatments:

1. Harpin (1:60) (13  $\mu\text{gm/ml}$ ).
2. Harpin (1:90) (8.7  $\mu\text{gm/ml}$ ).
3. Buffer (5mM  $\text{KPO}_4$ , pH 6.8).



- 39 -

Table 6 - Mean Height of Tomato Plants after Treatment With Harpin.

5	Operation and Treatment			Mean height (cm) of tomato plants	
	Day 0	Day 14	Day 28	Day 42	Day 47
10	sowing	transplant	harpin 1:60 (13 $\mu$ gm/ml)	35.5	36.0
	sowing	transplant	harpin 1:90 (8.7 $\mu$ gm/ml)	35.7	36.5
15	sowing	transplant	buffer	32.5	33.0

As shown in Table 6, spraying tomato seedlings with *Erwinia amylovora* hypersensitive response elicitor can increase growth of tomato plants. Similar increases in growth were noted for the two doses of the

5 hypersensitive response elicitor tested compared with the buffer-treated control.

10 **Example 4 - Effect of Treating Tomato Seeds with *Erwinia amylovora* Hypersensitive Response Elicitor on Tomato Plant Height**

*Marglobe* tomato seeds were submerged in *Erwinia amylovora* hypersensitive response elicitor solution ("harpin") (1:40, 1:80, 1:160, 1:320, and 1:640) or

15 buffer in beakers on day 0 for 24 hours at 28°C in the growth chamber. After soaking seeds in harpin or buffer, they were sown in germination pots with artificial soil on day 1. Ten uniform appearing plants per treatment were chosen randomly and measured. The seedlings were

20 measured by ruler from the surface of soil to the top of plant.

- 40 -

**Treatments:**

1. Harpin (1:40) (20  $\mu\text{gm/ml}$ ).
2. Harpin (1:80) (10  $\mu\text{gm/ml}$ ).
3. Harpin (1:160) (5  $\mu\text{gm/ml}$ ).
4. Harpin (1:320) (2.5  $\mu\text{gm/ml}$ ).
5. Harpin (1:640) (1.25  $\mu\text{gm/ml}$ ).
6. Buffer (5mM  $\text{KPO}_4$ , pH 6.8).

Table 7 - Seedling Height (cm) 12 Days After Seed Treatment.

Treat	Plants	1	2	3	4	5	6	7	8	9	10	Mean
20 $\mu$ gm/ml	10	6.5	6.8	6.8	6.5	6.4	6.4	6.8	6.4	6.8	6.6	6.6
10 $\mu$ gm/ml	10	6.8	6.2	6.6	6.4	6.8	6.8	6.6	6.4	6.8	6.4	6.6
5 $\mu$ gm/ml	10	6.2	6.6	6.0	6.6	6.4	6.2	6.6	6.2	6.0	6.6	6.3
2.5 $\mu$ gm/ml	10	6.4	6.2	6.6	6.0	6.2	6.4	6.0	6.0	6.2	6.2	6.2
1.25 $\mu$ gm/ml	10	6.2	6.2	6.0	6.4	6.0	6.0	6.4	6.2	6.4	6.2	6.2
Buffer	10	5.8	6.0	6.2	6.2	5.8	5.8	6.0	6.2	6.0	6.0	6.0

Table 8 - Seedling Height (cm) 14 Days After Seed Treatment.

Treat	Plants	1	2	3	4	5	6	7	8	9	10	Mean
20 $\mu$ gm/ml	10	7.8	7.8	8.2	8.0	8.2	8.4	7.8	8.4	7.6	7.8	8.0
10 $\mu$ gm/ml	10	8.6	8.8	8.4	9.2	8.4	8.6	7.8	7.8	8.4	8.4	8.4
5 $\mu$ gm/ml	10	9.8	9.2	9.8	9.6	9.2	9.4	8.6	9.2	9.0	8.6	9.2
2.5 $\mu$ gm/ml	10	8.8	8.6	8.6	8.4	7.8	8.6	8.4	9.0	8.0	7.8	8.4
1.25 $\mu$ gm/ml	10	8.4	7.8	8.4	8.0	8.6	8.4	8.0	8.2	8.4	8.2	8.2
Buffer	10	7.2	8.2	7.4	7.6	7.8	7.6	7.8	7.4	7.8	7.6	7.6

Table 9 - Seedling Height (cm) 17 Days After Seed Treatment.

Treat	Plants	1	2	3	4	5	6	7	8	9	10	Mean
20 $\mu$ gm/ml	10	11.2	11.6	11.4	11.6	11.4	11.2	11.8	11.4	11.8	11.6	11.5
10 $\mu$ gm/ml	10	13.4	13.4	13.8	13.2	13.4	12.6	12.4	13.4	13.2	13.4	13.2
5 $\mu$ gm/ml	10	13.6	12.8	13.6	13.2	14.2	13.8	12.6	13.4	13.8	13.6	13.5
2.5 $\mu$ gm/ml	10	11.6	12.4	12.4	11.8	11.6	12.2	12.6	11.8	12.0	11.6	12.0
1.25 $\mu$ gm/ml	10	12.8	12.6	12.0	12.4	11.6	11.8	12.2	11.4	11.2	11.4	11.9
Buffer	10	10.0	10.4	10.6	10.6	10.4	10.4	10.8	10.2	10.4	10.0	10.4

Table 10 - Summary - Mean Height of Tomato Plants After Treatment

Operation and Treatment	Mean height of tomato plants (cm)					
	Day 0	Day 1	Day 12	Day 14	Day 17	
Harpin seed soak (20 $\mu$ gm/ml)	sowing	sowing	6.6	8.0	11.5	
Harpin seed soak (10 $\mu$ gm/ml)	sowing	sowing	6.6	8.4	13.2	
Harpin seed soak (5 $\mu$ gm/ml)	sowing	sowing	6.3	9.2	13.5	
Harpin seed soak (2.5 $\mu$ gm/ml)	sowing	sowing	6.2	8.4	12.0	
Harpin seed soak (1.25 $\mu$ gm/ml)	sowing	sowing	6.2	8.2	11.9	
Buffer seed soak	sowing	sowing	6.0	7.6	10.4	

- 43 -

As shown in Tables 7-10, the treatment of tomato seeds with *Erwinia amylovora* hypersensitive response elicitor can increase growth of tomato plants. A 1:160 dilution (5  $\mu\text{g}/\text{ml}$  harpin) had the greatest effect -- seedling  
5 height was increased more than 20% over the buffer treated plants.

10 **Example 5 - Effect of Treating Tomato Seeds with *Erwinia amylovora* Hypersensitive Response Elicitor on Seed Germination Percentage**

Marglobe tomato seeds were submerged in 40ml of *Erwinia amylovora* hypersensitive response elicitor ("harpin") solution (dilutions of CFEP from *E. coli* DH5  
15 (pCPP2139) of 1:50 or 1:100 which contained, respectively, 8  $\mu\text{g}/\text{ml}$  and 4  $\mu\text{g}/\text{ml}$  of hypersensitive response elicitor) and buffer in beakers on day 0 for 24 hours at 28°C in a growth chamber. After soaking, the seeds were sown in germination pots with artificial soil  
20 on day 1. This treatment was carried out on 20 seeds per pot and 4 pots per treatment.

**Treatments:**

- 25 1. Harpin (8  $\mu\text{g}/\text{ml}$ ).
2. Harpin (8  $\mu\text{g}/\text{ml}$ ).
3. Harpin (8  $\mu\text{g}/\text{ml}$ ).
4. Harpin (8  $\mu\text{g}/\text{ml}$ ).
5. Harpin (4  $\mu\text{g}/\text{ml}$ ).
6. Harpin (4  $\mu\text{g}/\text{ml}$ ).
- 30 7. Harpin (4  $\mu\text{g}/\text{ml}$ ).
8. Harpin (4  $\mu\text{g}/\text{ml}$ ).
9. Buffer (5mM  $\text{KPO}_4$ , pH 6.8).
10. Buffer (5mM  $\text{KPO}_4$ , pH 6.8).
11. Buffer (5mM  $\text{KPO}_4$ , pH 6.8).
- 35 12. Buffer (5mM  $\text{KPO}_4$ , pH 6.8).

- 44 -

Table 11 - Number of Seedlings After Seed Treatment With Harpin

5	Operation and Treatment		Number of seeds germinated (out of a total of 20)					
			Day 0		Day 1		Day 5	
			Day 42		Day 47			
			Mean		Mean		Mean	
10	Harpin (8 $\mu$ gm/ml)	sowing	11		15		19	
	Harpin (8 $\mu$ gm/ml)	sowing	13		17		20	
	Harpin (8 $\mu$ gm/ml)	sowing	10		13		16	
	Harpin (8 $\mu$ gm/ml)	sowing	9	10.8	15	15.0	16	17.8
15	Harpin (4 $\mu$ gm/ml)	sowing	11		17		17	
	Harpin (4 $\mu$ gm/ml)	sowing	15		17		18	
	Harpin (4 $\mu$ gm/ml)	sowing	9		12		14	
	Harpin (4 $\mu$ gm/ml)	sowing	9	11.0	14	15.0	16	16.3
20	Buffer	sowing	11		11		14	
	Buffer	sowing	9		14		15	
	Buffer	sowing	10		14		14	
	Buffer	sowing	10	10.0	12	12.8	14	14.3

25

As shown in Table 11, treatment of tomato seeds with *Erwinia amylovora* hypersensitive response elicitor can increase germination rate and level of tomato seeds. The higher dose used appeared to be more effective than buffer at the end of the experiment.

Example 6 - Effect on Plant Growth of Treating Tomato Seeds with Proteins Prepared from *E. coli* Containing a Hypersensitive Response Elicitor Encoding Construct, pCPP2139, or Plasmid Vector pCPP50

Marglobe tomato seeds were submerged in *Erwinia amylovora* hypersensitive response elicitor ("harpin") (from *E. coli* DH5 $\alpha$ (pCPP2139) (Figure 1) or vector preparation (from DH5 $\alpha$ (pCPP50) (Figure 2) with added BSA protein as control. The control vector preparation contained, per ml, 33.6  $\mu$ l of BSA (10 mg/ml) to provide about the same amount of protein as contained in the

- 45 -

pCPP2139 preparation due to harpin. Dilutions of 1:50 (8.0  $\mu\text{g/ml}$ ), 1:100 (4.0  $\mu\text{g/ml}$ ), and 1:200 (2.0  $\mu\text{g/ml}$ ) were prepared in beakers on day 1, and seed was submerged for 24 hours at 28°C in a controlled environment chamber.

- 5 After soaking, seeds were sown in germination pots with artificial soil on day 2. Ten uniform appearing plants per treatment were chosen randomly and measured at three times after transplanting. The seedlings were measured by ruler from the surface of soil to the top of plant.

10

**Treatments:**

- |    |                 |       |                         |
|----|-----------------|-------|-------------------------|
| 1. | Harpin          | 1:50  | (8.0 $\mu\text{g/ml}$ ) |
| 2. | Harpin          | 1:100 | (4.0 $\mu\text{g/ml}$ ) |
| 3. | Harpin          | 1:200 | (2.0 $\mu\text{g/ml}$ ) |
| 15 | 4. Vector + BSA | 1:50  | (0 harpin)              |
|    | 5. Vector + BSA | 1:100 | (0 harpin)              |
|    | 6. Vector + BSA | 1:200 | (0 harpin)              |

Table 12 - Seedling Height (cm) 18 Days After Seed Treatment

Treat	Harpin	1	2	3	4	5	6	7	8	9	10	Mean
H1:50	8.0	3.6	5.0	4.8	5.0	4.2	5.2	5.8	4.6	4.0	4.8	4.7
H1:100	4.0	4.6	5.8	6.2	6.0	5.6	6.8	6.0	4.8	5.6	6.2	5.8
H1:200	2.0	4.0	5.8	5.8	4.6	5.4	5.0	5.8	4.6	4.6	5.8	5.1
V1:50	0	3.8	5.0	4.6	5.4	5.6	4.6	5.0	5.2	4.6	4.8	4.9
V1:100	0	4.4	5.2	4.6	4.4	5.4	4.8	5.0	4.6	4.4	5.2	4.8
V1:200	0	4.2	4.8	5.4	4.6	5.0	4.8	4.8	5.4	4.6	5.0	4.9

Table 13 - Seedling Height (cm) 22 Days After Seed Treatment.

Treat	Harpin	1	2	3	4	5	6	7	8	9	10	Mean
H1:50	8.0	4.2	5.6	5.2	6.0	4.8	5.4	5.0	5.2	5.4	5.0	5.2
H1:100	4.0	7.6	6.8	7.0	7.2	6.8	7.4	7.6	7.0	6.8	7.4	7.2
H1:200	2.0	7.0	6.6	6.8	7.2	7.4	6.8	7.0	7.2	6.8	7.2	7.0
V1:50	0	5.6	5.8	6.2	6.4	5.6	5.2	5.6	5.8	6.0	5.8	5.8
V1:100	0	5.4	6.0	5.8	6.2	5.8	5.6	5.4	5.2	6.0	5.6	5.7
V1:200	0	5.2	6.2	5.8	5.4	6.2	6.0	5.6	6.4	5.8	6.0	5.9



Table 14 - Seedling Height (cm) 26 Days After Seed Treatment.

Treat.	Harpin	1	2	3	4	5	6	7	8	9	10	Mean
H1:50	8.0	7.6	8.4	8.8	6.8	9.6	8.2	7.4	9.8	9.2	9.0	8.5
H1:100	4.0	12.0	11.4	11.2	11.0	10.8	12.0	11.2	11.6	10.4	10.2	11.2
H1:200	2.0	10.6	11.2	11.6	10.2	11.0	10.8	10.0	11.8	10.2	10.6	10.8
V1:50	0	9.0	9.4	8.8	8.4	9.6	9.2	9.2	8.6	8.0	9.4	9.2
V1:100	0	9.2	10.0	9.8	9.6	8.4	9.4	9.6	9.8	8.0	9.6	9.3
V1:200	0	8.8	9.6	8.2	9.2	8.4	8.0	9.8	9.0	9.4	9.2	9.0

Table 15 - Mean Height of Tomato Plants After Treatment

Operation and Treatment		Mean height of tomato plants (cm)				
Day 1		Day 2	Day 18	Day 22	Day 26	
Harpin (1:50)	(8.0 $\mu$ gm/ml)	sowing	4.7	5.2	8.5	
Harpin (1:100)	(4.0 $\mu$ gm/ml)	sowing	5.8	7.2	11.2	
Harpin (1:200)	(2.0 $\mu$ gm/ml)	sowing	5.1	7.0	10.8	
Vector + BSA (1:50)	(0)	sowing	4.9	5.8	9.2	
Vector + BSA (1:100)	(0)	sowing	4.8	5.7	9.3	
Vector + BSA (1:200)	(0)	sowing	4.9	5.9	9.0	

- 48 -

As shown in Tables 12-15, treatment with *E. coli* containing the gene encoding the *Erwinia amylovora* hypersensitive response elicitor can increase growth of tomato plants. The 1:100 dilution (4.0  $\mu\text{g/ml}$ ) had the greatest effect, while higher and lower concentrations had less effect. Mean seedling height for treatment with 4.0  $\mu\text{g/ml}$  of harpin was increased about 20% relative to vector control preparation, which contained a similar amount of non-harpin protein. Components of the lysed cell preparation from the strain *E. coli* DH5 $\alpha$ (pCPP50), which harbors the vector of the *hrpN* gene in *E. coli* strain DH5 $\alpha$ (pCPP2139), do not have the same growth-promoting effect as the harpin-containing preparation, even given that it is supplemented with BSA protein to the same extent as the DH5 $\alpha$ (pCPP2139) preparation, which contains large amounts of harpin protein.

Example 7 - Effect on Tomato Plant Growth of Treating Tomato Seeds with Proteins Prepared from *E. coli* Containing a Hypersensitive Response Elicitor Encoding Construct, pCPP2139, or its Plasmid Vector pCPP50

Marglobe tomato seeds were submerged in *Erwinia amylovora* hypersensitive response elicitor solution ("harpin") (from the harpin encoding plasmid pCPP2139 vector) and from pCPP50 vector-containing solution at dilutions of 1:25, 1:50, and 1:100 in beakers on day 1 for 24 hours at 28°C in a growth chamber. After soaking seeds, they were sown in germination pots with artificial soil on day 2. Ten uniform appearing plants per treatment were chosen randomly and measured. The seedlings were measured by ruler from the surface of soil to the top of plant.

35

- 49 -

**Treatments:**

1. Harpin 16  $\mu\text{gm/ml}$
2. Harpin 8  $\mu\text{gm/ml}$
3. Harpin 4  $\mu\text{gm/ml}$
4. Vector 16  $\mu\text{gm/ml}$
5. Vector 8  $\mu\text{gm/ml}$
6. Vector 4  $\mu\text{gm/ml}$

Table 16 - Seedling Height (cm) 11 Days After Seed Treatment

Treat.	Harpin	Plants	1	2	3	4	5	6	7	8	9	10	Mean
H1:25	16 $\mu$ gm/ml	10	5.0	5.2	4.8	4.6	4.4	4.6	3.8	4.2	3.8	4.2	4.5
H1:50	8 $\mu$ gm/ml	10	5.6	5.4	6.0	5.8	4.8	6.8	5.8	5.0	5.2	4.8	5.5
H1:100	4 $\mu$ gm/ml	10	5.2	5.6	5.0	5.0	5.0	4.8	5.0	5.6	4.8	5.2	5.1
V1:25	0	10	4.4	4.4	4.8	4.6	4.8	4.6	4.0	4.8	4.4	4.6	4.5
V1:50	0	10	4.8	4.4	4.6	4.0	4.4	4.2	4.6	4.0	4.4	4.2	4.4
V1:100	0	10	4.6	4.2	4.8	4.4	4.4	4.0	4.2	4.0	4.4	4.0	4.3

Table 17 - Seedling Height (cm) 14 Days After Seed Treatment

Treat.	Harpin	Plants	1	2	3	4	5	6	7	8	9	10	Mean
H1:25	16 $\mu$ gm/ml	10	7.6	7.6	7.2	7.4	7.8	7.8	7.6	7.0	7.4	7.0	7.4
H1:50	8 $\mu$ gm/ml	10	8.5	8.2	8.4	7.6	7.8	8.4	8.6	9.0	7.6	8.2	8.2
H1:100	4 $\mu$ gm/ml	10	7.2	8.4	8.2	7.4	8.0	7.6	7.6	8.0	8.6	7.6	7.9
V1:25	0	10	6.8	6.4	7.8	6.6	6.6	6.8	7.4	6.0	6.4	6.4	6.7
V1:50	0	10	6.6	5.8	6.4	7.6	7.4	7.2	6.8	6.6	6.4	5.8	6.7
V1:100	0	10	6.2	6.0	6.8	6.6	6.4	5.8	6.6	7.0	5.8	6.4	6.4

- 51 -

Table 18 - Mean Height of Tomato Plants After Treatment.

5	Operation and Treatment	Mean height of tomato plants (cm)			
		Day 1	Day 2	Day 11	Day 14
10	Harpin seed soak (16 $\mu$ gm/ml)	sowing	4.5	7.4	
	Harpin seed soak (8 $\mu$ gm/ml)	sowing	5.5	8.2	
	Harpin seed soak (4 $\mu$ gm/ml)	sowing	5.1	7.9	
	Vector seed soak (16 $\mu$ gm/ml)	sowing	4.5	6.7	
	Vector seed soak (8 $\mu$ gm/ml)	sowing	4.4	6.7	
	Vector seed soak (4 $\mu$ gm/ml)	sowing	4.3	6.4	
15					

As shown in Tables 16-18, treatment with *Erwinia amylovora* hypersensitive response elicitor can increase growth of tomato plants. A 1:50 dilution (8  $\mu$ g/ml hypersensitive response elicitor) had the greatest effect with seedling height being increased by about 20% over the control.

**Example 8 - Effect of Cell-Free *Erwinia amylovora* Hypersensitive Response Elicitor on Growth of Potato**

Three-week-old potato plants, variety Norchip, were grown from tuber pieces in individual containers.

The foliage of each plant was sprayed with a solution containing *Erwinia amylovora* hypersensitive response elicitor ("harpin"), or a control solution containing proteins of *E. coli* and those of the vector pCPP50 ("vector"), diluted 1:50, 1:100, and 1:200. On day 20, 12 uniform appearing plants were chosen randomly for each of the following treatments. One plant from each treatment was maintained at 16°C, in a growth chamber, while two plants from each treatment were maintained on a greenhouse bench at 18-25°C. Twenty-five days after treatment, the shoots (stems) on all plants were measured individually.

- 52 -

**Treatments:**

1. Harpin 1:50	16 $\mu$ gm/ml
2. Harpin 1:100	8 $\mu$ gm/ml
3. Harpin 1:200	4 $\mu$ gm/ml
4. Vector 1:50	0 harpin
5. Vector 1:100	0 harpin
6. Vector 1:200	0 harpin

Table 19 - Length of Potato Stems of Plants at 16°C

Treatment on day 20	Length of potato stems (cm) on day 45					
	stem 1	stem 2	stem 3	stem 4	stem 5	Plant Mean
Harpin 1:50	43.0	39.5	42.5	34.0	38.0	39.4
Harpin 1:100	42.0	38.5	(2 branch)			40.3
Harpin 1:200	35.5	30.5	31.5	(3 branch)		32.5
Vector 1:50	34.0	32.0	31.5	28.0	27.5 (5 branch)	30.6
Vector 1:100	30.0	33.5	33.0	30.0	28.0	31.3
Vector 1:200	33.5	31.5	32.5	(3 branch)		32.5

Table 20 - Length of Potato Stems of Plants on a Greenhouse Bench

Treatment on day 20	Length of potato stems (cm) on day 45						Treat. Mean
	stem 1	stem 2	stem 3	stem 4	stem 5	Plant	
Harpin 1:50	65.5	58.5	57.5	62.5	68.5	62.5	64.2
Harpin 1:100	62.5	67.0	65.0	69.0	(4 branch)	65.9	
Harpin 1:200	70.5	73.5	74.0	80.5	(4 branch)	74.6	77.1
Vector 1:50	83.0	80.5	76.5	76.0	81.5 (5 branch)	79.5	
Vector 1:100	56.5	59.5	50.5	53.0	55.5	53.9	58.0
Vector 1:200	57.0	59.5	69.5	(3 branch)		62.0	
Vector 1:50	53.0	62.0	59.5	62.5 (4 branch)		59.3	57.6
Vector 1:100	52.0	46.0	61.5	56.5	61.5	55.8	
Vector 1:200	62.0	51.5	66.0	67.5	62.0	62.0	62.3
Vector 1:50	61.5	62.5	59.0	65.5	63.0	62.5	
Vector 1:100	62.0	66.0 (2 branch)				64.0	62.8
Vector 1:200	61.0	60.0	63.5 (3 branch)			61.5	

- 54 -

As shown in Tables 19 and 20, treatment of potato plants with *Erwinia amylovora* hypersensitive response elicitor enhanced shoot (stem) growth. Thus, overall growth, as judged by both the number and mean  
5 lengths of stems, were greater in the harpin-treated plants in both the greenhouse and growth chamber-grown plants. The potato plants treated with the medium dose of harpin (8  $\mu\text{gm/ml}$ ) seemed enhanced in their stem growth more than those treated with either higher or lower  
10 doses. Treatment with the medium dose of harpin resulted in greater growth under both growing conditions.

Example 9 - Effect of Spraying Tomatoes With a Cell-Free Elicitor Preparation Containing the  
15 *Erwinia amylovora* Harpin

Marglobe tomato plants were sprayed with harpin preparation (from *E. coli* DH5 $\alpha$ (pCPP2139)) or vector preparation (from *E. coli* DH5 $\alpha$ (pCPP50)) with added BSA  
20 protein as control 8 days after transplanting. The control vector preparation contained, per ml, 33.6  $\mu\text{l}$  of BSA (10 mg/ml) to provide about the same amount of protein as contained in the pCPP2139 preparation due to harpin. Dilutions of 1:50 (8.0  $\mu\text{g/ml}$ ), 1:100  
25 (4.0  $\mu\text{g/ml}$ ), and 1:200 (2.0  $\mu\text{g/ml}$ ) were prepared and sprayed on the plants to runoff with an electricity-powered atomizer. Fifteen uniform appearing plants per treatment were chosen randomly and assigned to treatment. The plants were maintained at 28°C in a controlled  
30 environment chamber before and after treatment.

Overall heights were measured several times after treatment from the surface of soil to the top of the plant. The tops of the tomato plants were weighed immediately after cutting the stems near the surface of  
35 the soil.



- 55 -

**Treatments:** (Dilutions and harpin content)

- |    |              |       |                  |
|----|--------------|-------|------------------|
| 1. | Harpin       | 1:50  | (8.0 $\mu$ g/ml) |
| 2. | Harpin       | 1:100 | (4.0 $\mu$ g/ml) |
| 3. | Harpin       | 1:200 | (2.0 $\mu$ g/ml) |
| 4. | Vector + BSA | 1:50  | (0 harpin)       |
| 5. | Vector + BSA | 1:100 | (0 harpin)       |
| 6. | Vector + BSA | 1:200 | (0 harpin)       |

Table 21 -Tomato plant height (cm) 1 day after spray treatment

Treat	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Mean
H 50	5.4	5.0	5.6	5.0	5.2	4.8	5.0	5.2	5.4	5.0	5.6	4.8	4.6	5.0	5.8	5.16
H 100	5.0	5.2	5.0	5.4	5.4	5.0	5.2	4.8	5.6	5.2	5.4	5.0	4.8	5.0	5.2	5.15
H 200	5.0	4.6	5.4	4.6	5.0	5.2	5.4	4.8	5.0	5.2	5.4	5.2	5.0	5.2	5.0	5.13
V 50	5.2	4.6	4.8	5.0	5.6	4.8	5.0	5.2	5.6	5.4	5.2	5.8	5.0	4.8	5.2	5.15
V 100	5.2	4.8	5.2	5.0	5.6	4.8	5.4	5.2	5.0	4.8	5.0	4.8	5.6	5.2	5.4	5.13
V 200	5.2	5.4	5.0	5.4	5.2	5.4	5.0	5.2	5.4	5.2	4.6	4.8	5.2	5.0	5.4	5.16

Table 22 -Tomato plant height (cm) 15 days after spray treatment

Treat	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Mean
H 50	22.0	21.0	22.0	21.5	23.0	22.0	23.5	25.0	22.0	20.5	21.0	23.5	22.0	22.5	21.0	22.2
H 100	26.0	26.5	27.0	29.0	27.5	26.0	28.0	29.0	28.5	26.0	27.5	28.0	28.0	29.0	26.0	27.5
H 200	24.5	26.0	25.0	26.0	26.5	27.5	28.5	28.0	26.0	24.0	26.5	24.5	26.0	24.0	27.5	26.0
V 50	23.5	21.5	20.5	22.5	20.5	21.0	22.0	23.5	22.0	20.5	22.0	21.0	20.5	22.5	21.5	21.7
V 100	22.5	21.0	20.5	23.0	22.0	20.0	20.5	20.0	21.0	22.0	23.0	20.0	22.0	21.0	22.5	21.4
V 200	21.5	20.5	23.5	20.5	22.0	22.0	22.5	20.0	22.0	23.5	23.5	22.0	20.0	23.0	21.0	21.8

Table 23 -Tomato plant height (cm) 21 days after spray treatment

Treat	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Mean
H 50	28.5	28.0	27.5	26.0	27.0	28.5	28.5	29.0	30.0	28.5	29.0	27.0	28.5	28.0	27.0	28.1
H 100	37.0	38.0	37.5	39.0	37.0	38.5	36.0	38.0	37.0	38.5	37.0	36.0	37.0	37.0	38.5	37.5
H 200	34.5	34.0	36.0	33.5	32.0	34.5	32.5	34.0	32.0	36.5	30.5	32.0	30.0	32.5	34.0	33.2
V 50	30.0	28.0	28.0	28.5	30.0	27.0	26.5	28.0	29.5	28.5	26.5	28.5	27.0	29.5	28.5	28.3
V 100	28.0	27.5	30.0	29.5	28.5	29.0	30.0	26.5	27.5	28.0	30.0	29.0	28.5	28.0	29.5	28.6
V 200	28.5	30.5	27.0	29.0	28.5	27.5	29.0	30.0	28.0	28.5	29.0	30.5	27.5	28.5	28.0	28.7

Table 24 -Mean Height of Tomato Plants After Spraying

Treatment (Dil. & harpin)	Mean height of tomato plants (cm)			
	Days After Treatment			
	Day 1	Day 11	Day 14	
Harpin 1:50	5.16	22.2	28.1	(8.0 µg/ml)
Harpin 1:100	5.15	27.5	37.5	(4.0 µg/ml)
Harpin 1:200	5.13	26.0	33.2	(2.0 µg/ml)
Vector + BSA 1:50	5.15	21.7	28.5	(0)
Vector + BSA 1:100	5.13	21.4	28.6	(0)
Vector + BSA 1:200	5.16	21.8	28.7	(0)

Table 25 - Fresh Weight of Tomato Plants (g/plant)  
21 Days After Spray Treatment

Treat	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Mean
H 50	65.4	60.3	58.9	73.2	63.8	70.1	58.4	60.1	62.7	55.6	58.3	68.9	58.2	64.2	56.4	62.3
H 100	84.3	68.8	74.6	66.7	78.5	58.9	76.4	78.6	84.8	78.4	86.4	66.5	76.5	82.4	80.5	76.2
H 200	80.1	76.5	68.4	79.5	64.8	79.6	76.4	80.2	66.8	72.5	78.8	72.3	62.8	76.4	73.2	73.9
V 50	64.0	56.8	69.4	72.3	56.7	66.8	71.2	62.3	61.0	62.5	63.4	58.3	72.1	67.8	67.0	64.7
V 100	62.8	58.4	70.2	64.2	58.1	72.7	68.4	53.6	67.5	66.3	59.3	68.2	71.2	65.2	59.2	64.4
V 200	64.2	59.6	70.2	66.6	64.3	60.4	60.8	56.7	71.8	60.6	63.6	58.9	68.3	57.2	60.0	62.9

- 59 -

A single spray of tomato seedlings with harpin, in general, resulted in greater subsequent growth than spray treatment with the control (vector) preparation, which had been supplemented with BSA protein. Enhanced growth in the harpin-treated plants was seen in both plant height and fresh weight measurements. Of the three concentrations tested, the two lower ones resulted in more plant growth (based on either measure) than the higher dose (8.0  $\mu\text{g/ml}$ ). There was little difference in the growth of plants treated with the two lower (2 and 4  $\mu\text{g/ml}$ ) concentrations. Components of the lysed cell preparation from the strain *E. coli* DH5 $\alpha$ (pCPP50), which harbors the vector of the *hrpN* gene in *E. coli* strain DH5 $\alpha$ (pCPP2139), do not have the same growth-promoting effect as the harpin-containing preparation, even though it is supplemented with BSA protein to the same extent as the DH5 $\alpha$ (pCPP2139) preparation, which contains large amounts of harpin protein. Thus, this experiment demonstrates that harpin is responsible for enhanced plant growth.

**Example 10 - Early Coloration and Early Ripening of Small Fruits**

A field trial was conducted to evaluate the effect of hypersensitive response elicitor ("harpin") treatment on yield and ripening parameters of raspberry cv. Canby. Established plants were treated with harpin at 2.5 mg/100 square feet in plots 40 feet long x 3 feet wide (1 plant wide), untreated ("Check"), or treated with the industry standard chemical Ronilan at recommended rates ("Ronilan"). Treatments were replicated four times and arranged by rep in an experimental field site. Treatments were made beginning at 5-10% bloom followed by two applications at 7-10 day intervals. The first two harvests were used to evaluate disease control and fruit

- 60 -

yield data was collected from the last two harvests. Observations indicated harpin-treated fruits were larger and exhibited more redness than untreated fruits, indicating ripening was accelerated by 1-2 weeks. The  
 5 number of ripe fruits per cluster bearing a minimum of ten fruits was determined at this time and is summarized in Table 26. Harpin treated plots had more ripe fruits per 10-berry cluster than either the check or Ronilan treatments. Combined yields from the last two harvests  
 10 indicated increased yield in harpin and Ronilan treated plots over the untreated control (Table 27).

Table 26 - Number of Ripe Raspberry Fruits Per Clusters With Ten Berries or More on June 20, 1996.

<u>Treatment</u>	<u>Ripe fruit/10 berry clusters</u>	<u>% of Control</u>
Check	2.75	100.0
Ronilan	2.75	100.0
Harpin	7.25	263.6

Table 27 - Mean Raspberry Fruit Yield by Weight (lbs.) Combined in Last Two Harvest.

<u>Treatment</u>	<u>Total Yield</u>	<u>% of Control</u>
Check	32.5	100.0
Ronilan	37.5	115.4
Harpin	39.5	121.5

#### Example 11 - Growth Enhancement For Snap Beans

Snap beans of the variety Bush Blue Lake were treated by various methods, planted in 25-cm-d plastic  
 5 pots filled with commercial potting mix, and placed in an open greenhouse for the evaluation of growth parameters. Treatments included untreated bean seeds ("Check"), seeds treated with a slurry of 1.5% methyl cellulose prepared with water as diluent ("M/C"), seeds treated with 1.5%  
 10 methyl cellulose followed by a foliar application of hypersensitive response elicitor ("harpin") at 0.125

- 61 -

mg/ml ("M/C+H"), and seeds treated with 1.5% methyl cellulose plus harpin spray dried at 5.0 µg harpin per 50 seeds followed by a foliar application of harpin at 0.125 mg/ml ("M/C-SD+H"). Seeds were sown on day 0, planted 3  
 5 per pot, and thinned to 1 plant per pot upon germination. Treatments were replicated 10 times and randomized by rep in an open greenhouse. Bean pods were harvested after 64 days, and fresh weights of bean pods of marketable size (>10 cm x 5 cm in size) were collected as yield. Data  
 10 were analyzed by analysis of variance with Fisher's LSD used to separate treatment means.

Table 28 - Effect of *Erwinia amylovora* Harpin Treatment  
 15 by Various Methods on Yield of Market Sized Snap Bean Pods

	<u>Treatment</u>	<u>Marketable Yield, g<sup>1</sup></u>	<u>% of Untreated (Check)</u>
20	M/C-SD+H	70.6 a	452
	M/C-H	58.5 ab	375
	M/C	46.3 bc	297
	M/C+H	42.3 bc	271
	M/C-SD	40.0 cd	256
25	Check	15.6 e	100

<sup>1</sup> Marketable yield included all bean pods 10 cm x 0.5 cm or larger. Means followed by the same letter are not significantly different at  
 30 P=0.05 according to Fisher's LSD.

As shown in Table 28, the application of *Erwinia amylovora* harpin by various methods of application  
 35 resulted in an increase in the yield of marketable size snap bean pods. Treatment with methyl cellulose alone also results in an increase in bean yield but was substantially increased when combined with harpin as seed (spray dried) and foliar treatments.

40

**Example 12 - Yield Increase in Cucumbers from Foliar Application of HP-1000™ to Cucumbers.**

Cucumber seedlings and transplants were treated  
 45 with foliar sprays of HP-1000™ (EDEN Bioscience, Bothell,

- 62 -

Washington) (*Erwinia amylovora* hypersensitive response elicitor formulation) at rates of 15, 30, or 60  $\mu\text{g/ml}$  active ingredient (a.i.). The first spray was applied when the first true leaves were fully expanded. The

5 second application was made 10 days after the first spray. All sprays were applied using a back-pack sprayer, and an untreated control (UTC) was also included in the trial. Three days after the second application of HP-1000™, ten plants from each treatment were

10 transplanted into randomized field plots replicated three times. This yielded a total of thirty plants per treatment. Seven days after transplanting, a third foliar spray of HP-1000™ was applied. Although severe drought followed resulting in significant water stress, a total

15 of six harvests were made following a standard commercial harvesting pattern. The total weight of fruit harvested from each treatment is presented in Table 29. Results indicate that plants treated with HP-1000™ at rates of 15 and 30  $\mu\text{g/ml}$  yielded significantly more fruit than the

20 UTC. Plants treated with HP-1000™ yielded a moderate yield increase. These results indicated that HP-1000™ treated plants were significantly more tolerant to drought stress conditions than untreated plants.

25 Table 29 - Increase yield of cucumbers after treatment with HP-1000™

30	Treatment	Rate <sup>1</sup>	Yield, <sup>2</sup> lbs./10 plants	% above UTC
	UTC	---	9.7 a	---
	HP-1000™	15 $\mu\text{g/ml}$	25.4 b	161.4
	HP-1000™	30 $\mu\text{g/ml}$	32.6 c	236.4
	HP-1000™	60 $\mu\text{g/ml}$	11.2 a	15.9

35 -----

<sup>1</sup>Active ingredient (a.i.). <sup>2</sup>Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

40



- 63 -

**Example 13 - Yield Increase in Cotton from Treatment with HP-1000™**

Cotton was planted in four, 12 x 20 foot replicate field plots in a randomized complete block (RCB) field trial. Plants were treated with HP-1000™ (EDEN Bioscience) (*Erwinia amylovora* hypersensitive response elicitor formulation), HP-1000™+Pix° (Pix° (BASF Corp., Mount Olive, N.J.) is a growth regulator applied to keep cotton plants compact in height) or Early Harvest° (Griffen Corp., Valdosta, Ga.) (a competitive growth enhancing agent). An untreated control (UTC) was also included in the trial. Using a back-pack sprayer, foliar applications were made of all treatments at three crop growth stages; first true leaves, pre-bloom, and early bloom. All fertilizers and weed control products were applied according to conventional farming practices for all treatments. The number of cotton bolls per plant ten weeks before harvest was significantly higher for the HP-1000™ treated plants compared to other treatments. By harvest, HP-1000™ treatment was shown to have a significantly increased lint yield (43%) compared to UTC (Table 30). When HP-1000™ was combined with Pix°, lint yield was increased 20% over UTC. Since Pix° is commonly applied to large acreages of cotton, this result indicates that HP-1000™ may be successfully tank-mixed with Pix°. Application of the competitive growth enhancing agent, Early Harvest° only produced a 9% increase in lint yield vs. UTC.

30

- 64 -

Table 30 - Increased lint yield from cotton after treatment with HP-1000™, HP-1000™+Pix®, or Early Harvest®.

5	-----
	Treatment              Rate <sup>1</sup> Lint Yield (lbs./ac)              % above
	UTC
	UTC              ---              942.1              ---
10	Early Harvest®    2 oz./ac.              1,077.4*              14.3
	HP-1000™+Pix®    40 µg/ml+8 oz./ac.    1,133.1*              20.4
	HP-1000™              40 µg/ml              1,350.0*              43.3
	(*significant at P= 0.05)              lsd = 122.4
15	-----

<sup>1</sup>Rates for HP-1000™ are for active ingredient (a.i.); rates for Early Harvest® and Pix® are formulated product.

20

**Example 14 - Yield Increase of Chinese Egg Plant from Treatment with HP-1000™**

Nursery grown Chinese egg plant seedlings were  
 25 sprayed once with HP-1000™ at (EDEN Bioscience) (Erwinia amylovora hypersensitive response elicitor formulation) 15, 30, or 60 µg/ml (a.i.), then transplanted into field plots replicated three times for each treatment. Two weeks after transplanting, a second application of  
 30 HP-1000™ was made. A third and final application of HP-1000™ was applied approximately two weeks after the second spray. All sprays were applied using a back-pack sprayer; an untreated control (UTC) was also included in the trial. As the season progressed, a total of eight  
 35 harvests from each treatment were made. Data from these harvests indicate that treatment with HP-1000™ resulted in greater yield of fruit per plant.

40

- 65 -

Table 31 - Increased yield for Chinese egg plant after treatment with HP-1000™.

5	Treatment	Rate (a.i.)	Yield(lbs./plant)	% above UTC
	UTC	--	1.45	---
	HP-1000™	15 µg/ml	2.03	40.0
	HP-1000™	30 µg/ml	1.90	31.0
10	HP-1000™	60 µg/ml	1.95	34.5

15 Example 15 - Yield Increase of Rice From Treatment with HP-1000™

Rice seedlings were transplanted into field plots replicated three times, then treated with foliar sprays of HP-1000™ (EDEN Bioscience) (*Erwinia amylovora* hypersensitive response elicitor formulation) at three different rates using a back-pack sprayer. An untreated control (UTC) was also included in the trial. The first application of HP-1000™ was made one week after transplanting, the second three weeks after the first. A third and final spray was made just before rice grains began to fill the heads. Results at harvest demonstrated that foliar applications of HP-1000™ at both 30 and 60 µg/ml significantly increased yield by 47 and 56%, respectively (Table 32).

- 66 -

Table 32 - Increase yield of rice after foliar treatment with HP-1000™.

5	Treatment	Rate (a.i.)	Yield <sup>1</sup> (lbs./ac.)	% above UTC
	UTC	---	3,853 a	---
	HP-1000™	15 µg/ml	5,265 ab	35.9
	HP-1000™	30 µg/ml	5,710 b	47.3
10	HP-1000™	60 µg/ml	6,043 b	56.1

15 <sup>1</sup>Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

Example 16 - Yield Increase of Soybeans From Treatment with HP-1000™

20 Soybeans were planted into randomized field plots replicated three times for each treatment. A back-pack sprayer was used to apply foliar sprays of HP-1000™ (EDEN Bioscience) (*Erwinia amylovora*

25 hypersensitive response elicitor formulation) and an untreated control (UTC) was also included in the trial. Three rates of HP-1000™ were applied beginning at four true leaves when plants were approximately eight inches tall. A second spray of HP-1000™ was applied ten days

30 after the first spray and a third spray ten days after the second. Plant height measured ten days after the first spray treatment indicated that application of HP-1000™ resulted in significant growth enhancement (Table 33). In addition, plants treated with HP-1000™ at

35 the rate of 60 µg/ml began to flower five days earlier than the other treatments. Approximately ten days after application of the third spray, the number of soybean pods per plant was counted from ten randomly selected plants per replication. These results indicated that the

40 growth enhancement from treatment with HP-1000™ resulted in significantly greater yield (Table 34).

Table 33 - Increased plant height of soybeans after foliar treatment with HP-1000™.

5	Treatment	Rate (a.i.)	Plant Ht. <sup>1</sup> (in.)	% above UTC
	UTC	---	12.2 a	---
	HP-1000™	15 µg/ml	13.2 b	8.3
	HP-1000™	30 µg/ml	14.1 c	16.2
10	HP-1000™	60 µg/ml	14.3 c	17.3

<sup>1</sup>Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

Table 34 - Increased pod set of soybeans after foliar treatment with HP-1000™.

	Treatment	Rate (a.i.)	No. Pods/plant <sup>1</sup>	% above UTC
25	UTC	---	41.1 a	---
	HP-1000™	15 µg/ml	45.4 ab	10.4
	HP-1000™	30 µg/ml	47.4 b	15.4
	HP-1000™	60 µg/ml	48.4 b	17.7

<sup>1</sup>Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

#### Example 17 - Yield Increase of Strawberries From Treatment with HP-1000™

Two field trials with HP-1000™ (EDEN Bioscience) (Erwinia amylovora hypersensitive response elicitor formulation) were conducted on two strawberry varieties, Camarosa and Selva. For each variety, a randomized complete block (RCB) design was established having four replicate plots (5.33 x 10 feet) per treatment in a commercially producing strawberry field. Within each plot, strawberry plants were planted in a double row layout. An untreated control (UTC) was also included in the trial. Before applications began, all plants were picked clean of any flowers and berries.

- 68 -

Sprays of HP-1000™ at the rate of 40 µg/ml were applied as six weekly using a back-pack sprayer. Just prior to application of each spray, all ripe fruit from each treatment was harvested, weighed, and graded according to commercial standards. Within three weeks of the first application of HP-1000™ to *Selva* strawberry plants, growth enhancement was discernible as visibly greater above-ground biomass and a more vigorous, greener and healthier appearance. After six harvests (i.e. the scheduled life-span for these plants), all yield data were summed and analyzed. For the *Camarosa* variety, yield of marketable fruit from HP-1000™ treated plants was significantly increased (27%) over the UTC when averaged over the last four pickings (Table 35). Significant differences between treatments were not apparent for this variety for the first two pickings. The *Selva* variety was more responsive to the growth enhancing effects from treatment with HP-1000™; *Selva* strawberry plants yielded a statistically significant 64% more marketable fruit vs. the UTC when averaged over six pickings (Table 35).

Table 35 - Increased yield of strawberries after foliar treatment with HP-1000™.

	Treatment	Rate (a.i.)	Yield <sup>1</sup> (lbs./rep)		% above
30	UTC				
	Variety: <i>Camarosa</i>				
	UTC	---	1.71	a	---
	HP-1000™	40 µg/ml	2.17	b	27
	Variety: <i>Selva</i>				
35	UTC	---	0.88	a	---
	HP-1000™	40 µg/ml	1.44	b	64

<sup>1</sup>Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

- 69 -

**Example 18 - Earlier Maturity and Increased Yield of Tomatoes from Treatment with HP-1000™**

Fresh market tomatoes (var. *Solar Set*) were grown in plots (2 x 30 feet) replicated 5 times in a randomized complete block (RCB) field trial within a commercial tomato production field. Treatments included HP-1000™ (EDEN Bioscience) (*Erwinia amylovora* hypersensitive response elicitor formulation), an experimental competitive product (Actigard™ (Novartis, Greensboro, N.C.)) and a chemical standard (Kocide® (Griffen Corp., Valdosta, GA)) + Maneb® (DuPont Agricultural Products, Wilmington, D.E.)) for disease control. The initial application of HP-1000™ was made as a 50 ml drench (of 30 µg/ml a.i.) poured directly over the seedling immediately after transplanting. Thereafter, eleven weekly foliar sprays were applied using a back-pack sprayer. The first harvest from all treatments was made approximately six weeks after transplanting and only fully red, ripe tomatoes were harvested from each treatment. Results indicated that HP-1000™ treated plants had a significantly greater amount of tomatoes ready for the first harvest (Table 36). The tomatoes harvested from the HP-1000™ treated plants were estimated to be 10-14 days ahead other treatments.

- 70 -

Table 36 - Increased yield of tomatoes at first harvest after foliar treatment with of HP-1000™.

5	Treatment	Rate (a.i.) <sup>1</sup>	Yield <sup>2</sup> (lbs./rep)	% above
	UTC			
	UTC	---	0.61 a	---
	HP-1000™	30 µg/ml	2.87 b	375
10	Actigard™	14 g/ac	0.45 a	-25.1
	Kocide®	2 lbs./ac.	0.31 a	-49.1
	Maneb®	1 lb./ac		

15 <sup>1</sup>Rates for Kocide® and Maneb® are for formulated product. <sup>2</sup>Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

20

Example 19 - Earlier Flowering and Growth Enhancement of Strawberries From Treatment with HP-1000™ When Planted in Non-fumigated Soil.

25

Strawberry plants ("plugs" and "bare-root"), cv. *Commander* were transplanted into plots (2 x 30 feet) replicated 5 times in a randomized complete block field trial. Approximately sixty individual plants were transplanted into each replicate. Treatments applied in this field trial are listed below:

35	<u>Treatment</u>	<u>Application method</u>
	HP-1000™ (plug plants)	50-ml drench solution of HP-1000™ (EDEN Bioscience) ( <i>Erwinia amylovora</i> hypersensitive response elicitor formulation) at 40 µg/ml (a.i.) poured directly over the individual plants immediately after transplanting into non-fumigated soil <sup>1</sup> , followed by foliar applications of HP-1000™ at 40 µg/ml every 14 days.
40		
45	HP-1000™ 40 (bare-root plants)	root soak in solution of HP-1000™ at µg/ml (a.i.) for 1 hour, immediately before transplanting into non-fumigated



- 71 -

soil,<sup>1</sup> followed by foliar applications of HP-1000™ at 40 µg/ml every 14 days.

- |    |   |  |
|----|---|--|
| 5  | methyl bromide/<br>chlorpicrin<br>75/25 | soil fumigation at 300 lbs./ac via injection prior to transplanting, no HP-1000™ treatments applied. |
| 10 | Telone/chlorpicrin<br>70/30             | soil fumigation at 45 gal./ac via injection prior to transplanting, no HP-1000™ treatments applied.  |
| 15 | untreated control<br>(UTC)              | no fumigation, no HP-1000™ treatments  |
- <sup>1</sup>Non-fumigated soil had been cropped to vetch for the two previous years.
- 20 Transplanting was done in late fall when cool weather tended to slow plant growth. Two weeks after transplanting, the first foliar application of HP-1000™ was made at 40 µg/ml (a.i.) with a back-pack sprayer. Three weeks after transplanting, preliminary results were
- 25 gathered comparing HP-1000™ treatment against methyl bromide and UTC by counting the number of flowers on all strawberry "plug" plants in each replication. Since flowering had not yet occurred in the "bare-root" plants, each plant in replicates for this treatment was assessed
- 30 for early leaf growth by measuring the distance from leaf tip to stem on the middle leaf of 3-leaf cluster. Results (Tables 37 and 38) indicated that treatment with HP-1000™ provided early enhanced flower growth and leaf size for "plug" and "bare-root" strawberry plants,
- 35 respectively.

Table 37 - Earlier flowering of "plug" strawberry transplants after foliar treatment with HP-1000™.

	Treatment	Rate (a.i.)	No. flowers/rep <sup>1</sup>	% above
	UTC			
	UTC	---	2.0a	---
10	HP-1000™	40 µg/ml	7.5 b	275
	Methyl bromide/ chlorpicrin	300 lbs./ac	5.3 b	163

<sup>1</sup>Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

Table 38 - Increased leaf growth in "bare-root" strawberry transplants after foliar treatment with HP-1000™.

25	-----			
	Treatment	Rate (a.i.)	Leaf length <sup>1</sup> (in.)	% above
	UTC			
	UTC	---	1.26 a	---
30	HP-1000™	40 µg/ml	1.81 b	44

35 <sup>1</sup>Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

**Example 20 - Early Growth Enhancement of Jalapeño Peppers from Application of HP-1000™**

40 Jalapeño pepper (cv. *Mittlya*) transplants were  
treated with a root drench of HP-1000 (EDEN Bioscience)  
(*Erwinia amylovora* hypersensitive response elicitor  
formulation) (30 µg/ml a.i.) for 1 hour, then  
45 transplanted into randomized field plots replicated four  
times. An untreated control (UTC) was also included.  
Beginning 14 days after transplanting, treated plants  
received three foliar sprays of HP-1000™ at 14 day

- 73 -

intervals using a back-pack sprayer. One week after the third application of HP-1000™ (54 days after transplanting), plant height was measured from four randomly selected plants per replication. Results from these measurements indicated that the HP-1000™ treated plants were approximately 26% taller than the UTC plants (Table 39). In addition, the number of buds, flowers or fruit on each plant was counted. These results indicated that the HP-1000™ treated plants had over 61% more flowers, fruit or buds compared to UTC plants (Table 40).

Table 39 - Increased plant height in Jalapeño peppers after treatment with HP-1000™.

	Treatment	Rate (a.i.)	Plant Ht. (in.) <sup>1</sup>	% above UTC
	UTC	---	a7.0	---
20	HP-1000™	30 µg/ml	8.6 b	23.6

<sup>1</sup>Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

Table 40 - Increased number of flowers, fruit or buds in Jalapeño peppers after treatment with HP-1000™.

	Treatment	Rate (a.i.)	No. flowers, fruit or buds/plant <sup>1</sup>	% above
	UTC	---	20.6 a	---
40	HP-1000™	30 µg/ml	12.8 b	61.3

<sup>1</sup>Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

- 74 -

**Example 21 - Growth Enhancement of Tobacco from Application of HP-1000™**

Tobacco seedlings were transplanted into randomized field plots replicated three times. A foliar spray of HP-1000™ (EDEN Bioscience) (*Erwinia amylovora* hypersensitive response elicitor formulation) was applied after transplanting at one of three rates: 15, 30, or 60 µg/ml a.i. Sixty days later, a second foliar application of HP-1000 was made. Two days after the second application, plant height, number of leaves per plant, and the leaf size (area) were measured from ten, randomly selected plants per treatment. Results from these measurements indicated treatment with HP-1000™ enhanced tobacco plant growth significantly (Tables 41, 42, and 43). Plant height was increased by 6-13%, while plants treated with HP-1000™ at 30 and 60 µg/ml averaged just over 1 more leaf per plant than UTC. Most significantly, however, treatment with HP-1000™ at 15, 30, and 60 µg/ml resulted in corresponding increases in leaf area. Tobacco plants with an extra leaf per plant and an increase in average leaf size (area) represent a commercially significant response.

Table 41 - Increased plant height in tobacco after treatment with HP-1000™.

Treatment	Rate (a.i.)	Plant Ht. (cm)	% above UTC
UTC	---	72.0	---
HP-1000™	15 µg/ml	76.4	5.3
HP-1000™	30 µg/ml	79.2	9.0
HP-1000™	60 µg/ml	81.3	6.9

- 75 -

Table 42 - Increased number of tobacco leaves per plant after treatment with HP-1000™.

5	Treatment	Rate (a.i.)	Leaves/plant <sup>1</sup>	% above UTC
	UTC	---	16.8	---
	HP-1000™	15 µg/ml	17.4	3.6
	HP-1000™	30 µg/ml	18.1	7.7
10	HP-1000™	60 µg/ml	17.9	6.5

Table 43 - Increased leaf area in tobacco after treatment with HP-1000™.

20	Treatment	Rate (a.i.)	Leaf area (cm <sup>2</sup> )	% above UTC
	UTC	---	1,246	---
	HP-1000™	15 µg/ml	1,441	16
	HP-1000™	30 µg/ml	1,543	24
25	HP-1000™	60 µg/ml	1,649	32

### 30 Example 22 - Growth Enhancement of Winter Wheat from Application of HP-1000™

Winter wheat seed was "dusted" with dry HP-1000™ (EDEN Bioscience) (*Erwinia amylovora* hypersensitive response elicitor formulation) powder at the rate of 3 ounces of formulated product (3% a.i.) per 100 lbs. seed, then planted using conventional seeding equipment into randomized test plots 11.7 feet by 100 feet long. Additional treatments included a seed "dusting" with HP-1000™ powder (3% a.i.) at 1 oz. formulated product per 100 lbs. seed, a seed-soak in a solution of HP-1000™ at a concentration of 20 µg/ml, a.i., for four hours, then air-dried before planting, a standard chemical (Dividend®) fungicide "dusting", and an untreated control (UTC). Eight days after planting,

- 76 -

HP-1000™ treated seeds began to emerge, whereas the UTC and chemical standard-treated seed did not emerge until approximately 14 days after planting, the normal time expected. At 41 days after planting, seedlings were removed from the ground and evaluated. Root mass for wheat treated with HP-1000™ as a "dusting" at 3 oz./100 lb. was visually inspected and judged to be approximately twice as great as any of the other treatments.

Following the field trial, a greenhouse experiment was designed to gain confirmation of these results. Treatments included wheat seed dusted with dry HP-1000™ (10% a.i.) at a rate of 3 ounces per 100 lbs. of seed, seed soaking of HP-1000™ in solution concentration of 20 mg/ml for four hours before planting, and an untreated control (UTC). Wheat seeds from each treatment were planted at the rate of 25 seeds per pot, with five pots serving as replicates for each treatment. Fifteen days after planting, ten randomly selected seedlings from each treatment pot were removed, carefully cleaned, and measured for root length. Since the above-ground portion of individual seedlings did not exhibit any treatment effect, increased root growth from treatment with HP-1000™ did not influence the selection of samples. The increase in root growth from either HP-1000™ treatment was significantly greater than UTC (Table 49); however, the seed dusting treatment appeared to give slightly better results.

- 77 -

Table 44 - Increased root growth in wheat seedlings after treatment with HP-1000™.

5	-----			
	Treatment	Rate	Root length. (cm) <sup>1</sup>	% above UTC
	UTC	---	35.6 a	---
	HP-1000™			
	(dusting)	3 oz./100 lbs.	41.0 b	17.4
10	HP-1000™			
	(soaking)	20 µg/ml	40.8 b	14.6
	-----			

15 <sup>1</sup>Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

20 **Example 23 - Growth Enhancement of Cucumbers from Application of HP-1000™**

A field trial of commercially produced cucumbers consisted of four treatments, HP-1000™ (EDEN Bioscience) (*Erwinia amylovora* hypersensitive response elicitor formulation) at two rates (20 or 40 µg/ml), a chemical standard for disease control (Bravo® (Zeneca Ag Products, Wilmington, Del.) +Maneb®) and an untreated control (UTC). Each treatment was replicated four times in 3 x 75 foot plots with a plant spacing of approximately 2 feet for each treatment. Foliar sprays of HP-1000™ were applied beginning at first true leaf and repeated at 14 day intervals until the last harvest for a total of six applications. The standard fungicide mix was applied every seven days or sooner if conditions warranted. Commercial harvesting began approximately two months after first application of HP-1000™ (after five sprays of HP-1000™ had been applied), and a final harvest was made approximately 14 days after the first harvest.

40 Results from the first harvest indicated that treatment with HP-1000™ enhanced the average cucumber yield by increasing the total number of cucumbers

- 78 -

harvested and not the average weight of individual cucumbers (Tables 45-47). The same trend was noted at the final harvest (Tables 48-49). It was commercially important that the yield increase resulting from treatment with HP-1000™ was not achieved by significantly increasing average cucumber size.

Table 45 - Increased cucumber yield after treatment with HP-1000™, first harvest.

Treatment	Rate (a.i.)	Yield/trt <sup>1</sup> (kg.)	% above UTC
UTC	---	10.0 a	---
Bravo+Maneb	label	10.8 a	8.4
HP-1000™	20 µg/ml	12.3 ab	22.8
HP-1000™	40 µg/ml	13.8 b	38.0

<sup>1</sup>Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

Table 46 - Increased number of fruit in cucumbers after treatment with HP-1000™, first harvest.

Treatment	Rate (a.i.)	No. fruit/trt <sup>1</sup>	% above UTC
UTC	---	24.5 a	---
Bravo+Maneb	label	27.6 ab	12.8
HP-1000™	20 µg/ml	31.2 b	27.0
HP-1000™	40 µg/ml	34.3 b	39.8

<sup>1</sup>Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.



- 79 -

Table 47 - Average weight of cucumbers after treatment with HP-1000™, first harvest.

5	Treatment	Rate (a.i.)	Weight/fruit(g)	% change vs.
	UTC			
	UTC	---	406	---
	Bravo+Maneb	label	390	-4
10	HP-1000™	20 µg/ml	395	-3
	HP-1000™	40 µg/ml	403	-1

15

Table 48 - Increased cucumber yield after treatment with HP-1000™, third harvest.

20	Treatment	Rate (a.i.)	Yield/trt <sup>1</sup> (kg.)	% above UTC
	UTC	---	17.5 a	---
	Bravo+Maneb	label	14.0 b	-20.1
25	HP-1000™	20 µg/ml	20.1 a	15.3
	HP-1000™	40 µg/ml	20.2 a	15.6

30 <sup>1</sup>Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

35 Table 49 - Increased number of fruit in cucumbers after treatment with HP-1000™, third harvest.

40	Treatment	Rate (a.i.)	No. fruit/trt <sup>1</sup>	% change vs.
	UTC			
	UTC	---	68.8 ab	---
	Bravo+Maneb	label	60.0 a	-12.7
	HP-1000™	20 µg/ml	82.3 b	19.6
45	HP-1000™	40 µg/ml	85.3 b	24.0

50 <sup>1</sup>Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

Table 50 - Average weight of cucumbers after treatment with HP-1000™, third harvest.

5	Treatment	Rate (a.i.)	Weight/fruit(g)	% change vs. UTC
	UTC	---	255	---
	Bravo+Maneb	label	232	-9
10	HP-1000™	20 µg/ml	247	-3
	HP-1000™	40 µg/ml	237	-7

15  
**Example 24 - Harpin<sub>pss</sub> from *Pseudomonas syringae* pv *syringae* Induces Growth Enhancement in Tomato**

20 To test if harpin<sub>pss</sub> (i.e. the hypersensitive response elicitor from *Pseudomonas syringae* pv *syringae*) (He, S. Y., et al., "Pseudomonas syringae pv *syringae* Harpin<sub>pss</sub>. A Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 25 73:1255-66 (1993), which is hereby incorporated by reference) also stimulates plant growth, tomato seeds (Marglobe variety) were sowed in 8 inches pots with artificial soil. 10 days after sowing, the seedlings were transplanted into individual pots. Throughout the 30 experiment, fertilizer, irrigation of water, temperature, and soil moisture were maintained uniformly among plants. 16 days after transplanting, the initial plant height was measured and the first application of harpin<sub>pss</sub> was made, this is referred to as day 0. A second application was 35 made on day 15. Additional growth data was collected on day 10 and day 30. The final data collection on day 30 included both plant height and fresh weight.

The harpin<sub>pss</sub> used for application during the experiment was produced by fermenting *E. coli* DH5 40 containing the plasmid with the gene encoding harpin<sub>pss</sub> (i.e. *hrpZ*). The cells were harvested, resuspended in 5 mM potassium phosphate buffer, and disrupted by

- 81 -

sonication. The sonicated material was boiled for 5 minutes and then centrifugated for 10 min. at 10,000 rpm. The supernatant was considered as Cell-Free Elicitor Preparation (CFEP). 20 and 50 µg/ml harpin<sub>pss</sub> solution was made with the same buffer used to make cell suspension. CFEP prepared from the same strain containing the same plasmid but without *hrpZ* gene was used as the material for control treatment.

The wetting agent, Pinene II (Drexel Chemical Co., Memphis, Tenn.) was added to the harpin<sub>pss</sub> solution at the concentration of 0.1%, then harpin<sub>pss</sub> was sprayed onto tomato plant until there was run off.

Table 51 shows that there was a significant difference between the harpin<sub>pss</sub> treatment groups and the control group. Harpin<sub>pss</sub> treated tomato increased more than 10% in height. The data supports the claim that harpin<sub>pss</sub> does act similar to the hypersensitive response elicitor from *Erwinia amylovora*, in that when applied to tomato and many other species of plants, there is a growth enhancement effect. In addition to a significant increase of tomato height harpin<sub>pss</sub>-treated tomato had more biomass, big leaves, early flower setting, and over all healthier appearance.

Table 51 - Harpin<sub>pss</sub> enhances the growth of tomato plant

Treatment	Plant Height (cm <sup>1</sup> )			
	Day 0	Day 10	Day 30	
CFEP Control	8.5 <sup>2</sup> (0.87) a <sup>3</sup>	23.9 (1.90) a	68.2	(8.60) a
Harpinpss 20 µg/ml	8.8 (0.98) a	27.3 (1.75) b	74.2	(6.38) b
Harpinpss 50 µg/ml	8.8 (1.13) a	26.8 (2.31) b	75.4	6.30) b

<sup>1</sup>Plant height was measured to the nearest 0.5 cm. Day 0 refers to the day the initial plant heights were recorded and the first application was made.

- 82 -

<sup>2</sup>Means are given with SD in parenthesis (n=20 for all treatment groups).

- 5 <sup>3</sup>Different letters (a and b) indicates significant differences (P 0.05) among means. Differences were evaluated by ANOVA followed by Fisher LSD.

10           Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.

- 83 -

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: Cornell Research Foundation, Inc.
- (ii) TITLE OF INVENTION: ENHANCEMENT OF GROWTH IN PLANTS
- (iii) NUMBER OF SEQUENCES: 10
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: Nixon, Hargrave, Devans & Doyle LLP
  - (B) STREET: Clinton Square, P.O. Box 1051
  - (C) CITY: Rochester
  - (D) STATE: New York
  - (E) COUNTRY: U.S.A.
  - (F) ZIP: 14603
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER:
  - (B) FILING DATE:
  - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER: US 60/036,048
  - (B) FILING DATE: 27-JAN-1997
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Goldman, Michael L.
  - (B) REGISTRATION NUMBER: 30,727
  - (C) REFERENCE/DOCKET NUMBER: 19603/1502
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: (716) 263-1304
  - (B) TELEFAX: (716) 263-1600

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 338 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: linear

- 84 -

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```

Met Gln Ile Thr Ile Lys Ala His Ile Gly Gly Asp Leu Gly Val Ser
1           5           10           15
Gly Leu Gly Ala Gln Gly Leu Lys Gly Leu Asn Ser Ala Ala Ser Ser
20           25           30
Leu Gly Ser Ser Val Asp Lys Leu Ser Ser Thr Ile Asp Lys Leu Thr
35           40           45
Ser Ala Leu Thr Ser Met Met Phe Gly Gly Ala Leu Ala Gln Gly Leu
50           55           60
Gly Ala Ser Ser Lys Gly Leu Gly Met Ser Asn Gln Leu Gly Gln Ser
65           70           75           80
Phe Gly Asn Gly Ala Gln Gly Ala Ser Asn Leu Leu Ser Val Pro Lys
85           90           95
Ser Gly Gly Asp Ala Leu Ser Lys Met Phe Asp Lys Ala Leu Asp Asp
100          105          110
Leu Leu Gly His Asp Thr Val Thr Lys Leu Thr Asn Gln Ser Asn Gln
115          120          125
Leu Ala Asn Ser Met Leu Asn Ala Ser Gln Met Thr Gln Gly Asn Met
130          135          140
Asn Ala Phe Gly Ser Gly Val Asn Asn Ala Leu Ser Ser Ile Leu Gly
145          150          155          160
Asn Gly Leu Gly Gln Ser Met Ser Gly Phe Ser Gln Pro Ser Leu Gly
165          170          175
Ala Gly Gly Leu Gln Gly Leu Ser Gly Ala Gly Ala Phe Asn Gln Leu
180          185          190
Gly Asn Ala Ile Gly Met Gly Val Gly Gln Asn Ala Ala Leu Ser Ala
195          200          205
Leu Ser Asn Val Ser Thr His Val Asp Gly Asn Asn Arg His Phe Val
210          215          220
Asp Lys Glu Asp Arg Gly Met Ala Lys Glu Ile Gly Gln Phe Met Asp
225          230          235          240
Gln Tyr Pro Glu Ile Phe Gly Lys Pro Glu Tyr Gln Lys Asp Gly Trp
245          250          255
Ser Ser Pro Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser Lys
260          265          270
Pro Asp Asp Asp Gly Met Thr Gly Ala Ser Met Asp Lys Phe Arg Gln
275          280          285
Ala Met Gly Met Ile Lys Ser Ala Val Ala Gly Asp Thr Gly Asn Thr
290          295          300

```

- 85 -

Asn Leu Asn Leu Arg Gly Ala Gly Gly Ala Ser Leu Gly Ile Asp Ala  
 305 310 315 320

Ala Val Val Gly Asp Lys Ile Ala Asn Met Ser Leu Gly Lys Leu Ala  
 325 330 335

Asn Ala

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2141 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CGATTTTACC	CGGGTGAACG	TGCTATGACC	GACAGCATCA	CGGTATTCGA	CACCGTTACG	60
GCGTTTATGG	CCGCGATGAA	CCGGCATCAG	GCGGCGCGCT	GGTCGCCGCA	ATCCGGCGTC	120
GATCTGGTAT	TTCAGTTTGG	GGACACCGGG	CGTGAACCTCA	TGATGCAGAT	TCAGCCGGGG	180
CAGCAATATC	CCGGCATGTT	GCGCACGCTG	CTCGCTCGTC	GTTATCAGCA	GGCGGCAGAG	240
TGCGATGGCT	GCCATCTGTG	CCTGAACGGC	AGCGATGTAT	TGATCCTCTG	GTGGCCGCTG	300
CCGTCGGATC	CCGGCAGTTA	TCCGCAGGTG	ATCGAACGTT	TGTTTGAACT	GGCGGGAATG	360
ACGTTGCCGT	CGCTATCCAT	AGCACCGACG	GCGCGTCCGC	AGACAGGGAA	CGGACGCGCC	420
CGATCATTA	GATAAAGGCG	GCTTTTTTTT	TTGCAAAACG	GTAACGGTGA	GGAACCGTTT	480
CACCGTCGGC	GTCACCTCAGT	AACAAGTATC	CATCATGATG	CCTACATCGG	GATCGGCGTG	540
GGCATCCGTT	GCAGATACTT	TTGCGAACAC	CTGACATGAA	TGAGGAAACG	AAATTATGCA	600
AATTACGATC	AAAGCGCACA	TCGGCGGTGA	TTTGGGCGTC	TCCGGTCTGG	GGCTGGGTGC	660
TCAGGGACTG	AAAGGACTGA	ATTCCGCGGC	TTCATCGCTG	GGTTCCAGCG	TGGATAAACT	720
GAGCAGCACC	ATCGATAAGT	TGACCTCCGC	GCTGACTTCG	ATGATGTTTG	GCGGCGCGCT	780
GGCGCAGGGG	CTGGGCGCCA	GCTCGAAGGG	GCTGGGGATG	AGCAATCAAC	TGGGCCAGTC	840
TTTCGGCAAT	GGCGCGCAGG	GTGCGAGCAA	CCTGCTATCC	GTACCGAAAT	CCGGCGGCGA	900
TGCGTTGTCA	AAAATGTTTG	ATAAAGCGCT	GGACGATCTG	CTGGGTCATG	ACACCGTGAC	960
CAAGCTGACT	AACCAGAGCA	ACCAACTGGC	TAATTCAATG	CTGAACGCCA	GCCAGATGAC	1020
CCAGGGTAAT	ATGAATGCGT	TCGGCAGCGG	TGTGAACAAC	GCACTGTCGT	CCATTCTCGG	1080
CAACGGTCTC	GGCCAGTCGA	TGAGTGCTT	CTCTCAGCCT	TCTCTGGGGG	CAGGCGGCTT	1140

- 86 -

GCAGGGCCTG AGCGGCGCGG GTGCATTCAA CCAGTTGGGT AATGCCATCG GCATGGGCGT	1200
GGGGCAGAAT GCTGCGCTGA GTGCGTTGAG TAACGTCAGC ACCCACGTAG ACGGTAACAA	1260
CCGCCACTTT GTAGATAAAG AAGATCGCGG CATGGCGAAA GAGATCGGCC AGTTTATGGA	1320
TCAGTATCCG GAAATATTCG GTAAACCGGA ATACCAGAAA GATGGCTGGA GTTCGCCGAA	1380
GACGGACGAC AAATCCTGGG CTAAAGCGCT GAGTAAACCG GATGATGACG GTATGACCGG	1440
CGCCAGCATG GACAAATTCC GTCAGGCGAT GGGTATGATC AAAAGCGCGG TGGCGGGTGA	1500
TACCGGCAAT ACCAACCTGA ACCTGCGTGG CGCGGGCGGT GCATCGCTGG GTATCGATGC	1560
GGCTGTCGTC GGCATAAAA TAGCCAACAT GTCGCTGGGT AAGCTGGCCA ACGCCTGATA	1620
ATCTGTGCTG GCCTGATAAA GCGGAAACGA AAAAAGAGAC GGGGAAGCCT GTCTCTTTTC	1680
TTATTATGCG GTTTATGCGG TTACCTGGAC CGGTTAATCA TCGTCATCGA TCTGGTACAA	1740
ACGCACATTT TCCCGTTCAT TCGCGTCGTT ACGCGCCACA ATCGCGATGG CATCTTCCTC	1800
GTCGCTCAGA TTGCGCGGCT GATGGGGAAC GCCGGGTGGA ATATAGAGAA ACTCGCCGGC	1860
CAGATGGAGA CACGTCTGCG ATAAATCTGT GCCGTAACGT GTTCTATCC GCCCCTTTAG	1920
CAGATAGATT GCGGTTTCGT AATCAACATG GTAATGCGGT TCCGCCTGTG CGCCGGCCGG	1980
GATCACCACA ATATTCATAG AAAGCTGTCT TGCACCTACC GTATCGCGGG AGATACCGAC	2040
AAAATAGGGC AGTTTTTGCG TGGTATCCGT GGGGTGTTCC GGCCTGACAA TCTTGAGTTG	2100
GTTCGTCATC ATCTTTCTCC ATCTGGGCGA CCTGATCGGT T	2141

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 403 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met	Ser	Leu	Asn	Thr	Ser	Gly	Leu	Gly	Ala	Ser	Thr	Met	Gln	Ile	Ser
1				5					10					15	
Ile	Gly	Gly	Ala	Gly	Gly	Asn	Asn	Gly	Leu	Leu	Gly	Thr	Ser	Arg	Gln
			20					25					30		
Asn	Ala	Gly	Leu	Gly	Gly	Asn	Ser	Ala	Leu	Gly	Leu	Gly	Gly	Gly	Asn
			35				40					45			
Gln	Asn	Asp	Thr	Val	Asn	Gln	Leu	Ala	Gly	Leu	Leu	Thr	Gly	Met	Met
	50					55						60			



- 87 -

Met Met Met Ser Met Met Gly Gly Gly Gly Leu Met Gly Gly Gly Leu  
 65 70 75 80  
 Gly Gly Gly Leu Gly Asn Gly Leu Gly Gly Ser Gly Gly Leu Gly Glu  
 85 90 95  
 Gly Leu Ser Asn Ala Leu Asn Asp Met Leu Gly Gly Ser Leu Asn Thr  
 100 105 110  
 Leu Gly Ser Lys Gly Gly Asn Asn Thr Thr Ser Thr Thr Asn Ser Pro  
 115 120 125  
 Leu Asp Gln Ala Leu Gly Ile Asn Ser Thr Ser Gln Asn Asp Asp Ser  
 130 135 140  
 Thr Ser Gly Thr Asp Ser Thr Ser Asp Ser Ser Asp Pro Met Gln Gln  
 145 150 155 160  
 Leu Leu Lys Met Phe Ser Glu Ile Met Gln Ser Leu Phe Gly Asp Gly  
 165 170 175  
 Gln Asp Gly Thr Gln Gly Ser Ser Ser Gly Gly Lys Gln Pro Thr Glu  
 180 185 190  
 Gly Glu Gln Asn Ala Tyr Lys Lys Gly Val Thr Asp Ala Leu Ser Gly  
 195 200 205  
 Leu Met Gly Asn Gly Leu Ser Gln Leu Leu Gly Asn Gly Gly Leu Gly  
 210 215 220  
 Gly Gly Gln Gly Gly Asn Ala Gly Thr Gly Leu Asp Gly Ser Ser Leu  
 225 230 235 240  
 Gly Gly Lys Gly Leu Gln Asn Leu Ser Gly Pro Val Asp Tyr Gln Gln  
 245 250 255  
 Leu Gly Asn Ala Val Gly Thr Gly Ile Gly Met Lys Ala Gly Ile Gln  
 260 265 270  
 Ala Leu Asn Asp Ile Gly Thr His Arg His Ser Ser Thr Arg Ser Phe  
 275 280 285  
 Val Asn Lys Gly Asp Arg Ala Met Ala Lys Glu Ile Gly Gln Phe Met  
 290 295 300  
 Asp Gln Tyr Pro Glu Val Phe Gly Lys Pro Gln Tyr Gln Lys Gly Pro  
 305 310 315 320  
 Gly Gln Glu Val Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser  
 325 330 335  
 Lys Pro Asp Asp Asp Gly Met Thr Pro Ala Ser Met Glu Gln Phe Asn  
 340 345 350  
 Lys Ala Lys Gly Met Ile Lys Arg Pro Met Ala Gly Asp Thr Gly Asn  
 355 360 365  
 Gly Asn Leu Gln Ala Arg Gly Ala Gly Gly Ser Ser Leu Gly Ile Asp  
 370 375 380  
 Ala Met Met Ala Gly Asp Ala Ile Asn Asn Met Ala Leu Gly Lys Leu  
 385 390 395 400  
 Gly Ala Ala

- 88 -

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1288 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

AAGCTTCGGC ATGGCACGTT TGACCGTTGG GTCGGCAGGG TACGTTTGAA TTATTCATAA	60
GAGGAATACG TTATGAGTCT GAATACAAGT GGGCTGGGAG CGTCAACGAT GCAAATTTCT	120
ATCGGCGGTG CGGGCGGAAA TAACGGGTTG CTGGGTACCA GTCGCCAGAA TGCTGGGTTG	180
GGTGGCAATT CTGCACTGGG GCTGGGCGGC GGTAAATCAA ATGATACCGT CAATCAGCTG	240
GCTGGCTTAC TCACCGGCAT GATGATGATG ATGAGCATGA TGGGCGGTGG TGGGCTGATG	300
GGCGGTGGCT TAGGCGGTGG CTTAGGTAAT GGCTTGGGTG GCTCAGGTGG CCTGGGCGAA	360
GGACTGTCTG ACGCGCTGAA CGATATGTTA GGCGGTTTCG TGAACACGCT GGGCTCGAAA	420
GGCGGCAACA ATACCACTTC AACAACAAAT TCCCCGCTGG ACCAGGCGCT GGGTATTAAC	480
TCAACGTCCC AAAACGACGA TTCCACCTCC GGCACAGATT CCACCTCAGA CTCCAGCGAC	540
CCGATGCAGC AGCTGCTGAA GATGTTGAGC GAGATAATGC AAAGCCTGTT TGGTGATGGG	600
CAAGATGGCA CCCAGGGCAG TTCCTCTGGG GGCAAGCAGC CGACCGAAGG CGAGCAGAAC	660
GCCTATAAAA AAGGAGTCAC TGATGCGCTG TCGGGCCTGA TGGGTAATGG TCTGAGCCAG	720
CTCCTTGGA ACGGGGGACT GGGAGGTGGT CAGGGCGGTA ATGCTGGCAC GGGTCTTGAC	780
GGTTCGTCGC TGGGCGGCAA AGGGCTGCAA AACCTGAGCG GGCCGGTGGA CTACCAGCAG	840
TTAGGTAACG CCGTGGGTAC CGGTATCGGT ATGAAAGCGG GCATTCAGGC GCTGAATGAT	900
ATCGGTACGC ACAGGCACAG TTCAACCCGT TCTTTCGTCA ATAAAGGCGA TCGGGCGATG	960
GCGAAGGAAA TCGGTCAGTT CATGGACCAG TATCCTGAGG TGTTTGCAA GCCGCAGTAC	1020
CAGAAAGGCC CGGGTCAGGA GGTGAAAACC GATGACAAAT CATGGGCAA AGCACTGAGC	1080
AAGCCAGATG ACGACGGAAT GACACCAGCC AGTATGGAGC AGTTCAACAA AGCCAAGGGC	1140
ATGATCAAAA GGCCCATGGC GGGTGATACC GGCAACGGCA ACCTGCAGGC ACGCGGTGCC	1200
GGTGGTTCTT CGTGGGTAT TGATGCCATG ATGGCCGGTG ATGCCATTAA CAATATGGCA	1260
CTTGGCAAGC TGGGCGCGGC TTAAGCTT	1288

- 89 -

## (2) INFORMATION FOR SEQ ID NO:5:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 341 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```

Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met
 1              5              10              15

Ala Leu Val Leu Val Arg Pro Glu Ala Glu Thr Thr Gly Ser Thr Ser
 20              25              30

Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met
 35              40              45

Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala
 50              55              60

Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Gly Ile Glu Asp Val
 65              70              75              80

Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe
 85              90              95

Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met
100              105              110

Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu
115              120              125

Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met
130              135              140

Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro
145              150              155              160

Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe
165              170              175

Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile
180              185              190

Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly
195              200              205

Thr Gly Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser
210              215              220

Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser
225              230              235              240

Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp
245              250              255

```

- 90 -

Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Gly Leu Gly Thr Pro Val  
                                 260                                265                                270  
 Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln  
                                 275                                280                                285  
 Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Leu Lys Gly Leu Glu Ala  
                                 290                                295                                300  
 Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala  
                                 305                                310                                315                                320  
 Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg  
                                 325                                330                                335  
 Asn Gln Ala Ala Ala  
                                 340

## (2) INFORMATION FOR SEQ ID NO:6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1026 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATGCAGAGTC TCACTCTTAA CAGCAGCTCG CTGCAAACCC CGGCAATGGC CCTTGTCTCTG	60
GTACGTCTCTG AAGCCGAGAC GACTGGCAGT ACGTCGAGCA AGGCGCTTCA GGAAGTTGTC	120
GTGAAGCTGG CCGAGGAACT GATGCGCAAT GGTCAACTCG ACGACAGCTC GCCATTGGGA	180
AAACTGTTGG CCAAGTCGAT GGCCGCAGAT GGCAAGGCGG GCGGCGGTAT TGAGGATGTC	240
ATCGCTGCGC TGGACAAGCT GATCCATGAA AAGCTCGGTG ACAACTTCGG CGCGTCTGCG	300
GACAGCGCCT CGGGTACCGG ACAGCAGGAC CTGATGACTC AGGTGCTCAA TGGCCTGGCC	360
AAGTCGATGC TCGATGATCT TCTGACCAAG CAGGATGGCG GGACAAGCTT CTCCGAAGAC	420
GATATGCCGA TGCTGAACAA GATCGCGCAG TTCATGGATG ACAATCCCGC ACAGTTTCCC	480
AAGCCGGACT CGGGCTCCTG GGTGAACGAA CTCAAGGAAG ACAACTTCCT TGATGGCGAC	540
GAAACGGCTG CGTTCCGTTC GGCACCTGAC ATCATTGGCC AGCAACTGGG TAATCAGCAG	600
AGTGACGCTG GCAGTCTGGC AGGGACGGGT GGAGGTCTGG GCACTCCGAG CAGTTTTTCC	660
AACAACCTCGT CCGTGATGGG TGATCCGCTG ATCGACGCCA ATACCGGTCC CGGTGACAGC	720
GGCAATACCC GTGGTGAAGC GGGGCAACTG ATCGGCGAGC TTATCGACCG TGGCCTGCAA	780
TCGGTATTGG CCGGTGGTGG ACTGGGCACA CCCGTAAACA CCCCAGCAGAC CGGTACGTCTG	840

- 91 -

GCGAATGGCG GACAGTCCGC TCAGGATCTT GATCAGTTGC TGGGCGGCTT GCTGCTCAAG 900  
 GGCTTGGAGG CAACGCTCAA GGATGCCGGG CAAACAGGCA CCGACGTGCA GTCGAGCGCT 960  
 GCGCAAATCG CCACCTTGCT GGTCAGTACG CTGCTGCAAG GCACCCGCAA TCAGGCTGCA 1020  
 GCCTGA 1026

## (2) INFORMATION FOR SEQ ID NO:7:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 344 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln  
 1 5 10 15  
 Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser  
 20 25 30  
 Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile  
 35 40 45  
 Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly  
 50 55 60  
 Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala  
 65 70 75 80  
 Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser  
 85 90 95  
 Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met  
 100 105 110  
 Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala  
 115 120 125  
 Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val  
 130 135 140  
 Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala  
 145 150 155 160  
 Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly Gly  
 165 170 175  
 Gly Ala Gly Ala Gly Gly Ala Gly Gly Val Gly Gly Ala Gly Gly  
 180 185 190

- 92 -

Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Gly Gly Ala Asn Gly Ala  
 195 200 205  
 Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn  
 210 215 220  
 Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp  
 225 230 235 240  
 Gln Gly Gly Leu Thr Gly Val Leu Gln Lys Leu Met Lys Ile Leu Asn  
 245 250 255  
 Ala Leu Val Gln Met Met Gln Gln Gly Gly Leu Gly Gly Gly Asn Gln  
 260 265 270  
 Ala Gln Gly Gly Ser Lys Gly Ala Gly Asn Ala Ser Pro Ala Ser Gly  
 275 280 285  
 Ala Asn Pro Gly Ala Asn Gln Pro Gly Ser Ala Asp Asp Gln Ser Ser  
 290 295 300  
 Gly Gln Asn Asn Leu Gln Ser Gln Ile Met Asp Val Val Lys Glu Val  
 305 310 315 320  
 Val Gln Ile Leu Gln Gln Met Leu Ala Ala Gln Asn Gly Gly Ser Gln  
 325 330 335  
 Gln Ser Thr Ser Thr Gln Pro Met  
 340

## (2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1035 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

ATGTCAGTCG GAAACATCCA GAGCCCGTCG AACCTCCCGG GTCTGCAGAA CCTGAACCTC	60
AACACCAACA CCAACAGCCA GCAATCGGGC CAGTCCGTGC AAGACCTGAT CAAGCAGGTC	120
GAGAAGGACA TCCTCAACAT CATCGCAGCC CTCGTGCAGA AGGCCGCACA GTCGGCGGGC	180
GGCAACACCG GTAACACCGG CAACGCGCCG GCGAAGGACG GCAATGCCAA CGCGGGCGCC	240
AACGACCCGA GCAAGAACGA CCCGAGCAAG AGCCAGGCTC CGCAGTCGGC CAACAAGACC	300
GGCAACGTCG ACGACGCCAA CAACCAGGAT CCGATGCAAG CGCTGATGCA GCTGCTGGAA	360
GACCTGGTGA AGCTGCTGAA GGCGGCCCTG CACATGCAGC AGCCCGGCGG CAATGACAAG	420
GGCAACGGCG TGGGCGGTGC CAACGGCGCC AAGGGTGCCG GCGGCCAGGG CGGCCTGGCC	480

- 93 -

```

GAAGCGCTGC AGGAGATCGA GCAGATCCTC GCCCAGCTCG GCGGCGGCGG TGCTGGCGCC      540
GGCGGCGCGG GTGGCGGTGT CGGCGGTGCT GGTGGCGCGG ATGGCGGCTC CGGTGCGGGT      600
GGCGCAGGCG GTGCGAACGG CGCCGACGGC GGCAATGGCG TGAACGGCAA CCAGGCGAAC      660
GGCCCGCAGA ACGCAGGCGA TGTCAACGGT GCCAACGGCG CGGATGACGG CAGCGAAGAC      720
CAGGGCGGCC TCACCGGCGT GCTGCAAAAG CTGATGAAGA TCCTGAACGC GCTGGTGACG      780
ATGATGCAGC AAGGCGGCCT CGGCGGCGGC AACCAGGCGC AGGGCGGCTC GAAGGGTGCC      840
GGCAACGCCT CGCCGGCTTC CGGCGCGAAC CCGGGCGCGA ACCAGCCCGG TTCGGCGGAT      900
GATCAATCGT CCGGCCAGAA CAATCTGCAA TCCCAGATCA TGGATGTGGT GAAGGAGGTC      960
GTCCAGATCC TGCAGCAGAT GCTGGCGGCG CAGAACGGCG GCAGCCAGCA GTCCACCTCG     1020
ACGCAGCCGA TGTAAT                                     1035

```

## (2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 26 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```

Thr Leu Ile Glu Leu Met Ile Val Val Ala Ile Ile Ala Ile Leu Ala
1           5           10           15
Ala Ile Ala Leu Pro Ala Tyr Gln Asp Tyr
                20           25

```

## (2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 20 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

- 94 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Ser Ser Gln Gln Ser Pro Ser Ala Gly Ser Glu Gln Gln Leu Asp Gln  
1 5 10 15  
Leu Leu Ala Met  
20



## WHAT IS CLAIMED:

1. A method of enhancing growth in plants comprising:

applying a hypersensitive response elicitor polypeptide or protein in a non-infectious form to a plant or plant seed under conditions effective to enhance growth of the plant or plants grown from the plant seed.

2. A method according to claim 1, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from a pathogen selected from the group consisting of *Erwinia*, *Pseudomonas*, *Xanthomonas*, *Phytophthora*, and mixtures thereof.

3. A method according to claim 2, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Erwinia chrysanthemi*.

4. A method according to claim 2, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Erwinia amylovora*.

5. A method according to claim 2, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Pseudomonas syringae*.

6. A method according to claim 2, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Pseudomonas solanacearum*.

- 96 -

7. A method according to claim 2, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Xanthomonas campestris*.

8. A method according to claim 2, wherein the hypersensitive response elicitor polypeptide or protein corresponds to a *Phytophthora* species.

9. A method according to claim 1, wherein the plant is selected from the group consisting of dicots and monocots.

10. A method according to claim 9, wherein the plant is selected from the group consisting of rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

11. A method according to claim 9, wherein the plant is selected from the group consisting of rose, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

12. A method according to claim 1, wherein plants are treated during said applying which is carried out by spraying, injection, or leaf abrasion at a time proximate to when said applying takes place.

13. A method according to claim 1, wherein plant seeds are treated during said applying which is

- 97 -

carried out by spraying, injection, coating, dusting, or immersion.

14. A method according to claim 1, wherein the hypersensitive response elicitor polypeptide or protein is applied to plants or plant seeds as a composition further comprising a carrier.

15. A method according to claim 14, wherein the carrier is selected from the group consisting of water, aqueous solutions, slurries, and powders.

16. A method according to claim 14, wherein the composition contains greater than 0.5 nM of the hypersensitive response elicitor polypeptide or protein.

17. A method according to claim 14, wherein the composition further contains additives selected from the group consisting of fertilizer, insecticide, fungicide, nematocide, and mixtures thereof.

18. A method according to claim 1, wherein the hypersensitive response elicitor polypeptide or protein is in isolated form.

19. A method according to claim 1, wherein the hypersensitive response elicitor polypeptide or protein is applied as bacteria which do not cause disease and are transformed with a gene encoding the hypersensitive response elicitor polypeptide or protein.

20. A method according to claim 1, wherein the hypersensitive response elicitor polypeptide or protein is applied as bacteria which cause disease in some plant species, but not in those subjected to said applying, and

- 98 -

contain a gene encoding the hypersensitive response elicitor polypeptide or protein.

21. A method according to claim 1, wherein said applying causes infiltration of the polypeptide or protein into the plant.

22. A method according to claim 1, wherein said applying effects increased plant height.

23. A method according to claim 22, wherein plants are treated during said applying.

24. A method according to claim 22, wherein plant seeds are treated during said applying, said method further comprising:

planting the seeds treated with the hypersensitive response elicitor in natural or artificial soil and propagating the plants from the seeds planted in the soil.

25. A method according to claim 1, wherein plant seeds are treated during said applying to increase plant seed quantities which germinate, said method further comprising:

planting the seeds treated with the hypersensitive response elicitor protein or polypeptide in natural or artificial soil and

propagating plants from the seeds planted in the soil.

26. A method according to claim 1, wherein said applying effects greater yield.

- 99 -

27. A method according to claim 26, wherein plants are treated during said applying.

28. A method according to claim 26, wherein plant seeds are treated during said applying, said method further comprising:

planting the seeds treated with the hypersensitive response elicitor protein or polypeptide in natural or artificial soil and propagating plants from the seeds planted in the soil.

29. A method according to claim 1, wherein said applying effects earlier germination.

30. A method according to claim 29, wherein plant seeds are treated during said applying, said method further comprising:

planting the seeds treated with the hypersensitive response elicitor protein or polypeptide in natural or artificial soil and propagating plants from the seeds planted in the soil.

31. A method according to claim 29, wherein said applying effects earlier maturation.

32. A method according to claim 31, wherein plants are treated during said applying.

33. A method according to claim 31, wherein plant seeds are treated during said applying, said method further comprising:

- 100 -

planting the seeds treated with the  
hypersensitive response elicitor protein or polypeptide  
in natural or artificial soil and  
propagating plants from the seeds planted  
in the soil.

34. A method according to claim 1, wherein  
plant seeds are treated during said applying, said method  
further comprising:

planting the seeds treated with the  
hypersensitive response elicitor protein or polypeptide  
in natural or artificial soil and  
propagating plants from the seeds planted  
in the soil.

35. A method according to claim 34 further  
comprising:

applying the hypersensitive response  
elicitor protein or polypeptide in a non-infectious form  
to the propagated plants to enhance growth further.

36. A method according to claim 1, wherein  
said applying effects earlier fruit and plant coloration.

37. A method according to claim 36, wherein  
plant seeds are treated during said applying, said method  
further comprising:

planting the seeds treated with the  
hypersensitive response elicitor protein or polypeptide  
in natural or artificial soil and  
propagating plants from the seeds planted  
in the soil.

38. A method of enhancing growth in plants  
comprising:

- 101 -

providing a transgenic plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the transgenic plants or transgenic plants produced from the transgenic plant seeds under conditions effective to enhance plant growth.

39. A method according to claim 38, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from a pathogen selected from the group consisting of *Erwinia*, *Pseudomonas*, *Xanthomonas*, *Phytophthora*, and mixtures thereof.

40. A method according to claim 39, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Erwinia chrysanthemi*.

41. A method according to claim 39, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Erwinia amylovora*.

42. A method according to claim 39, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Pseudomonas syringae*.

43. A method according to claim 39, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Pseudomonas solanacearum*.

- 102 -

44. A method according to claim 39, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Xanthomonas campestris*.

45. A method according to claim 39, wherein the hypersensitive response eliciting polypeptide or protein corresponds to that derived from a *Phytophthora* species.

46. A method according to claim 38, wherein the plant is selected from the group consisting of dicots and monocots.

47. A method according to claim 46, wherein the plant is selected from the group consisting of rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

48. A method according to claim 46, wherein the plant is selected from the group consisting of rose, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

49. A method according to claim 38, wherein a transgenic plant is provided.

50. A method according to claim 38, wherein a transgenic plant seed is provided.



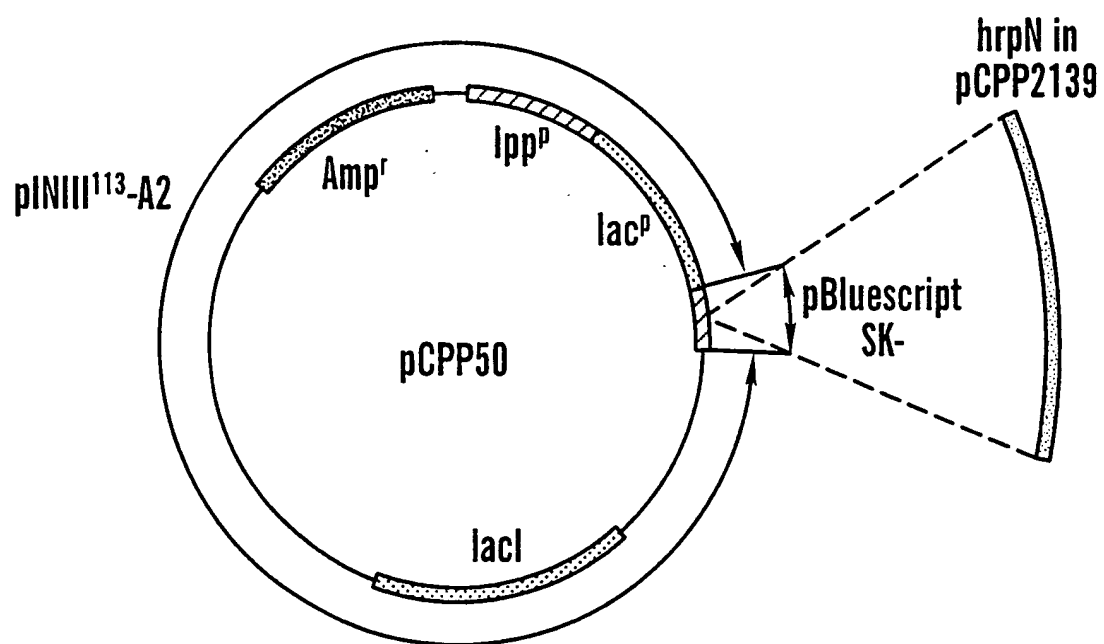
- 103 -

51. A method according to claim 38 further comprising:

applying the hypersensitive response elicitor polypeptide or protein to the propagated plants to enhance growth of the plant.

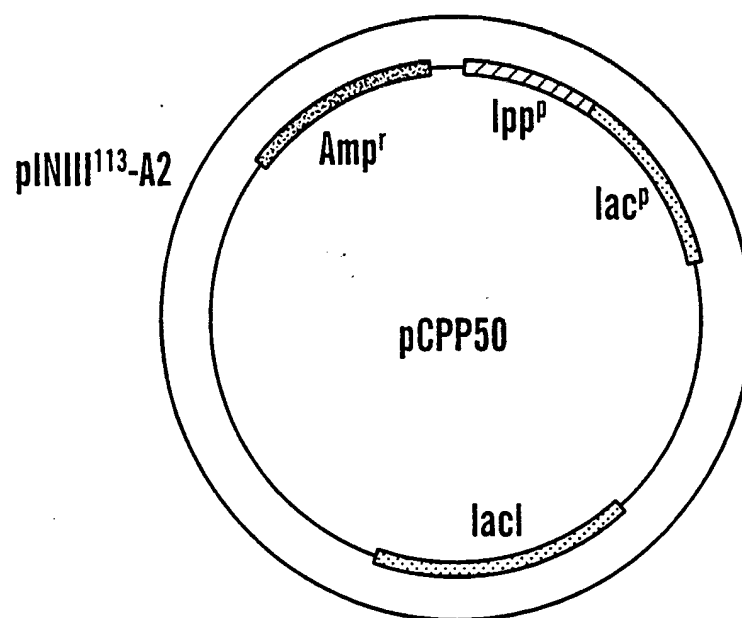


1/2

**FIG. 1**



2/2

**FIG. 2**



## INTERNATIONAL SEARCH REPORT

 International application No.  
PCT/US98/01507

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C12N 15/03, 15/05; A01N 13/00; A61K 39/00 1/11 1/15; A01H 4/00

US CL : 47/87; 435/252.3; 800/205

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 47/87; 435/252.3; 800/205

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Biosis, Medline, Wpids, NCBI, Agricola

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WEI et al. Harpin Elicitor of the Hypersensitive Response Produced by the Plant Pathogen Erwinia Amylovora. Science. July 1992, Vol. 257, pages 85-88. See entire reference.	1-18, 21-37
X ----- Y	BURR et al. Increase Potato Yields by Treatment of SeedPieces with Specific Strains of Pseudomonas fluorescens and P. putida. Phytopathology. September 1978, Vol. 68, pages, 1377-1383. See entire reference	1-2, 9-10-18, 21-37 ----- 1-18, 21-37
Y	KLOEPFER et al. Enhanced Plant Growth by Siderophores Produced by Plant Growth-Promoting Rhizobacteria. Nature. 28 August 1980, Vol. 286, 885-886. See entire reference.	1-18, 21-37

☐ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*B* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

09 MAY 1998

Date of mailing of the international search report

19 JUN 1998

 Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

Ousama M-Faiz Zaghmout

Telephone No. (703) 308-0196

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/01507

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:  
1-18,21-37
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐  
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.



## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/01507

### BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not linked as to form a single inventive concept under PCT Rule 13.1.

Group I. Claims 1-18, 21-37 are drawn to a method of enhancing growth in plants by applying the hypersensitive response elicitor polypeptide or protein in a non-infectious form to plants or seeds, classified in class 47, subclass 87 for example.

Group II. Claims 19-20 are drawn to a method wherein the hypersensitive response elicitor polypeptide or protein is applied as bacteria, classified in class 435, subclass 252.3 for example.

Group III. Claims 38-51 are drawn to a method for enhancing the growth of plant by producing transgenic or plant seed by transformation with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein, classified in class 800, subclass 205 for example.

The inventions listed as groups I, II and III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The invention of group I, drawn to a first process of making a first product, involves a method of enhancing the growth in plants by applying the hypersensitive response elicitor polypeptide or protein in a non-infectious form to plants or seeds not required by the groups II and III. The use of bacteria of invention in group II or transgenic plants of invention in group III are not required by the invention of group I.

The invention of group II involves a method wherein the hypersensitive response elicitor polypeptide or protein is applied as bacteria, not required by groups I where the response can be induced by protein which can be chemically synthesized, or by invention of group III where transgenic plants instead of bacteria.

The invention of group III to a method for enhancing the growth of plant by producing transgenic or plant seed by transformation with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein, not required by group I and II for the reasons specified above.

RECEIVED

7 1993

RECEIVED

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/01507

### BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not linked as to form a single inventive concept under PCT Rule 13.1.

Group I. Claims 1-18, 21-37 are drawn to a method of enhancing growth in plants by applying the hypersensitive response elicitor polypeptide or protein in a non-infectious form to plants or seeds, classified in class 47, subclass 87 for example.

Group II. Claims 19-20 are drawn to a method wherein the hypersensitive response elicitor polypeptide or protein is applied as bacteria, classified in class 435, subclass 252.3 for example.

Group III. Claims 38-51 are drawn to a method for enhancing the growth of plant by producing transgenic or plant seed by transformation with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein, classified in class 800, subclass 205 for example.

The inventions listed as groups I, II and III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The invention of group I, drawn to a first process of making a first product, involves a method of enhancing the growth in plants by applying the hypersensitive response elicitor polypeptide or protein in a non-infectious form to plants or seeds not required by the groups II and III. The use of bacteria of invention in group II or transgenic plants of invention in group III are not required by the invention of group I.

The invention of group II involves a method wherein the hypersensitive response elicitor polypeptide or protein is applied as bacteria, not required by groups I where the response can be induced by protein which can be chemically synthesized, or by invention of group III where transgenic plants instead of bacteria.

The invention of group III to a method for enhancing the growth of plant by producing transgenic or plant seed by transformation with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein, not required by group I and II for the reasons specified above.

RECEIVED

7 1998

1998